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SPONGOSPORA SUBTERRANEA AND PHOMA TUBEROSA ON THE IRISH POTATO

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INTRODUCTION

After finding that *Spongospora subterranea* was well established within our borders, the writers undertook to learn what its effect might be on the American potato (*Solanum tuberosum*) industry, taking up for this purpose the study of such questions as its geographical distribution and the factors governing the same, relation of the fungus to the roots and stems of the plant, and the possibility of its occurrence on other hosts. Such points as its damage to the tubers, relation to soil types, moisture, and control measures have received consideration. Some of these points have not been fully settled, but the data available are published here because of the widespread interest in this disease and its importance in many parts of the United States.

GEOGRAPHICAL DISTRIBUTION OF SPONGOSPORA SUBTERRANEA IN THE UNITED STATES

When *Spongospora subterranea* was discovered in Canada and in Maine, the question as to what would be its distribution in the United States immediately arose. Although quite extensive, European literature regarding the disease caused by this fungus contains but little information as to the factors that determine its geographical distribution. Owing to this fact and to the varied soil and climatic conditions in the United States, the writers were confronted with a new problem, which has been studied by means of surveys, by planting infected seed in many different localities, and by transplanting soil from various Southern States into the infected section of northern Maine.

DISTRIBUTION AS DETERMINED BY SURVEYS

Since the publication in the spring of 1914 of Bulletin 82 of the Department of Agriculture (8)[1], which showed that the disease was then known to

[1] Reference is made by number to "Literature cited," p. 253.

be generally distributed in northern and central Europe and the British Isles and was present in northeastern Canada and northern Maine, the writers, in cooperation with the Federal Horticultural Board and various State agencies, have extended the survey and as a result have found that the disease exists in isolated sections from the Atlantic to the Pacific. Table I gives its distribution in the United States.

TABLE I.—*Present distribution of powdery-scab in the United States*

First collection.		Collector.	Infected area.	Crop season.
Date.	Location.			
June 23, 1913	Presque Isle, Me....	I. E. Melhus.....	Aroostook County Washington County. Penobscot County	1912
Oct. 24, 1914	Chateaugay, N. Y..	.....do.....	Franklin County. Clinton County...	1914
Apr. 26, 1915	Nehalem, Oreg.....	F. D. Bailey.....	Clatsop County... Tillamook County	1914
May 1, 1915	Hastings, Fla.....	I. E. Melhus.....	St. Johns County.	1915
July 23, 1915	Snohomish, Wash...	F. D. Heald.....	Snohomish County.	1914
Sept. 18, 1915	Virginia, Minn.....	C. N. Frey..... E. C. Stakman.....	Carlton County... Lake County..... St. Louis County..	1915

As shown by Table I, there are six infested sections in the United States, all northern, except the one in Florida. This distribution is strikingly similar to that of *Phytophthora infestans* and lies wholly within its geographical range, which is confined to the northern part of the United States and to certain sections of the South in which a potato crop is grown during the winter.

DISTRIBUTION AS DETERMINED BY INFECTED SEED IN VARIOUS LOCALITIES

In order to determine whether *Spongospora subterranea* would develop south of infected sections in Maine and New York, infected seed was planted in 12 different States along the Atlantic seaboard in the spring of 1915, trials being made at more than one point in some States. The experiment was carried on in cooperation with the State pathologists and others interested in potato diseases, and the seed was planted in each case in accordance with the general practice in the respective locality. The results obtained are summarized in Table II.

As shown by Table II, no powdery-scab developed in any of these experiments. In each case all of the suspicious-looking specimens were sent to the writers for examination.

TABLE II.—*Results of planting seed infected with Spongospora subterranea outside the infected area*

Locality.	Grower.	Number of hills.	Result.
Arlington, Va.	Bureau of Plant Industry.	2,944	No powdery-scab.
West Raleigh, N. C.	H. R. Fulton	80	Do.
New Brunswick, N. J.	M. T. Cook	242	Do.
College Park, Md.	J. B. S. Norton	100	Do.
Gainesville, Fla.	H. E. Stevens	100	Do.
Ithaca, N. Y.	M. F. Barrus	72	Do.
East Marion, N. Y.	F. V. Rand	48	Do.
Geneva, N. Y.	F. C. Stewart	50	Do.
Newark, Del.	T. F. Manns	60	Do.
Amherst, Mass.	A. V. Osmun	18	Do.
Norfolk, Va.	T. C. Johnson	200	Do.
Clemson College, S. C.	H. W. Barre	100	Do.
New Haven, Conn.	G. P. Clinton	460	Do.
State College, Pa.	C. R. Orton	200	Do.
Morgantown, W. Va.	N. J. Giddings	81	Do.

As the season of 1915 was especially wet and still no *Spongospora subterranea* developed in any of the various localities named, it seems safe to assume that its chances for development in seasons with normal rainfall would be still more unfavorable.

Each of the trials referred to was on a small scale and for one season only, except that at Arlington, Va., which was carried through two seasons. In this trial 13 rows were planted in 1914 and 10 in 1915, or in all about 2,944 hills, with infected seed, and also, in 1915, 3 rows, or 384 hills, with clean seed in soil which had been planted with infected seed in 1914. In none of these rows was a single tuber found to be infected with *Spongospora subterranea;* consequently it seemed safe to assume that the conditions were not favorable to the disease, and this raised the question as to what constitutes the limiting conditions.

DISTRIBUTION AS DETERMINED BY TRANSPLANTING SOIL FROM OTHER STATES INTO NORTHERN MAINE

The influence of climate was studied by obtaining 200-pound lots of soil from 12 of the plots that had been planted with infected seed in nine of the States mentioned in Table II, shipping it to northern Maine, and planting it with infected seed of the Irish Cobbler variety. Naturally these soils varied materially in texture and composition, some being extremely light, while others were very heavy. These samples were placed in boxes 2 feet square and 8 inches deep, and the boxes set down into the soil in a field of virgin land where conditions were favorable for the development of *Spongospora subterranea.*

Because of the late arrival of most of the soils, planting was later than is usual in this section—that is, on June 19 in eight cases and on

July 3 in two cases—but otherwise the procedure was practically the same as that generally followed in northern Maine, and the plants received, so far as possible, the same cultural treatment as potatoes growing under field conditions. Owing to the late planting and early frost, the plants had a rather short growing season; and although not harvested until October 15, the tubers were small and in most cases immature. However, the conditions were sufficiently favorable to permit the development of the disease, which was the primary object of the experiment, and the results from this standpoint are given in Table III.

TABLE III.—*Results of planting potatoes infected with Spongospora subterranea at Presque Isle, Me., in soil from the plots in which diseased seed had been grown in various States.*

Source of soil.	Number of hills.	Number of sound tubers.	Tubers infected—	
			Number.	Per cent.
Diamond Springs, Va.	1	3	2	40.00
Amherst, Mass.	3	15	3	16.66
College Park, Md.	2	9	0	0
Morgantown, W. Va.	4	12	4	25.00
New Brunswick, N. J.	2	11	3	21.40
Unknown [a]	2	10	1	9.00
Geneva, N. Y.	1	8	0	0
Mount Carmel, Conn.	3	16	2	11.11
Gainesville, Fla.	2	14	1	6.66
Ithaca, N. Y.	2	8	3	27.27
Newark, Del.	2	15	0	0
Unknown [a]	1	7	0	0
Presque Isle, Me.	2	8	2	20.00

[a] In both of these cases the tags showing the source had been lost en route, which made it impossible to say definitely whence they came.

From the fact that *Spongospora subterranea* developed in 8 of the 12 soils tested, as shown by Table III, it seems safe to assume that it would have resulted in all had it been possible to have more soil and to make conditions more favorable for the growth and development of the potato plant. However, the experiment developed the important fact that powdery-scab was produced under Maine conditions in soils in which in their native States the disease was not produced.

This raised the question as to whether soil from infected sections will produce an infected crop when transplanted into a noninfected section. To test this, two lots of soil of 200 pounds each were collected from a field in northern Maine that produced an infected crop in 1914, and one was shipped to Washington, D. C., and was used in growing potatoes in the green house, and the other to Norfolk, Va., where it was placed in large iron cylinders sunk into the ground at the edge of a potato field and planted with clean tubers. The former produced a crop that became badly infected with powdery-scab, while the plants produced in the latter developed unusually well and the progeny re-

mained totally free from powdery-scab. This experiment indicates that when transplanted outside of the infected section infected soil does not yield an infected crop.

CLIMATIC CONDITIONS THE CONTROLLING FACTOR IN DISTRIBUTION

Unless climatic conditions are suitable, *Spongospora subterranea* will not develop, as is shown in the preceding pages. The studies in Maine show that periods of rainfall, followed by cool, damp, cloudy weather during the growing season, are periods of infection and are highly essential to the development of the disease. Such periods prevail during the growing season not only in the northern sections, but also in Florida, where potatoes are planted in January and February and harvested in April and May. Moreover, much of the infected area in Florida is in a section in which irrigation is practiced, the water there being supplied from artesian wells. The water level here is within a few inches of the surface for days at a time. Table IV shows the rainfall in the six infected areas during one growing season.

TABLE IV.—*Average monthly precipitation in powdery-scab-infected areas during the growing season of 1914 or 1915*

State.	Locality.	Period.	Average rainfall.
			Inches.
Florida	Hastings (St. Augustine).	February–July, 1915	4.20
Washington	Snohomish	April–October, 1914	2.50
Oregon	Astoria	do	3.64
Do	Glenora	May–October, 1914	4.70
Minnesota	Duluth	April–September, 1915	2.47
Do	Virginia	do	2.79
New York	Chateaugay (Dannemora).	April–October, 1914	2.80
Maine	Presque Isle	do	3.00
Do	do	April–October, 1915	3.08
Do	Van Buren	April–October, 1914	3.46
Do	do	April–September, 1915	3.38

PREVALENCE AND PERIOD OF EXISTENCE OF SPONGOSPORA SUBTERRANEA IN THIS COUNTRY

In Aroostook County, Me., *Spongospora subterranea* exists on many farms. In most cases the disease caused by this organism occurs in isolated spots, varying from a fraction of an acre to 5 acres; but in other places—for example, sections north and northwest of Caribou, including Perham, New Sweden, and Stockholm—infection is quite general. The disease is always most prevalent on wet, poorly drained land.

During the harvest season of 1914 several fields were examined; and notwithstanding the fact that fairly clean seed which had been treated with the usual strength of formaldehyde had been used, from 50 to 75 per

cent of the crop was found to be infected with *Spongospora subterranea*. In the case of two barrels of tubers selected at random from a 15-acre field near Caribou that had been planted to potatoes in 1914 after they had been picked by laborers and the healthy and infected tubers separated, 68 per cent by weight were found to be infected with the disease. The field in question was no exception, so far as the prevalence of the disease is concerned. Again, in the case of 117 hills which were dug at the same time by hand at random over 2 acres of this field and the infected and healthy tubers separated, 63 per cent of the progeny was infected and only two hills were free from the disease. It is needless to say that the crop thus infected could not be marketed for table use; and as the only way to turn it into money was to sell it for starch purposes, the grower sustained considerable loss, potatoes for starch usually selling for less than 15 cents per bushel.

The question as to how long land infected with *Spongospora subterranea* will remain contaminated is an interesting one. Pethybridge (12, p. 352), of Ireland, holds that it will remain infected for three years. In the case of two fields which, after growing a crop of potatoes, were in oats for one year and in meadow for four years and were planted to potatoes in 1914, over 50 per cent of the tubers were infected with *S. subterranea*, although there was every assurance that the seed used was free from the disease. Numerous other cases indicate that the disease can live in the soil for more than three years, and from facts at hand the writers believe that it can live for at least five years and probably much longer. It is evident, therefore, that when a piece of land once becomes infected, a very long rotation is necessary to rid it of infection and its value for growing potatoes is materially diminished.

The facts that *Spongospora subterranea* can live in the soil for at least five years and that it is prevalent in certain sections of Maine raise the question as to what length of time it has existed in this country. Its distribution in three counties of Maine, extending from the southern coast to the northern boundary, and in areas scattered over thousands of acres in the State naturally required some time; and consequently it must have existed in this country for a considerable period.

Not only is the disease widely distributed in Maine but also in the adjoining Province of New Brunswick, the St. John River Valley being quite generally infected, and in Prince Edward Island, which has long been settled. As the earlier settlers of northern Maine came from parts of New England farther south and up the St. John River Valley and as more than 60 per cent of the population of the State is of New Brunswick origin it is evident that over half the inhabitants of Maine came from a section generally infected with *Spongospora subterranea*. In view of this fact and that the disease exists in this country and in Canada, it seems reasonable to believe that it has existed in the infected sections of Maine for at least 15 years.

SUSCEPTIBILITY OF ROOTS, STOLONS, AND STEMS OF POTATO PLANTS TO SPONGOSPORA SUBTERRANEA

Although *Spongospora subterranea* is known only as a disease of the tuber of the Irish potato, the fact that its causal organism is a slime mold and that many other species of the family flourish on the root systems of their hosts led the writers to suspect that it infects the other underground parts of the potato. Very meager information on this phase of the subject was obtained from a survey of the literature on the disease. Johnson (5) incidentally mentions seeing pustules on stolons and Pethybridge (12, p. 352) mentions evidence of the disease on roots and sprouts, but in no way do these references show the prevalence and actual relationship. In the fall of 1914 a lot of 200 pounds of soil was collected in a field in which a crop of potatoes infected with *S. subterranea* had just been grown. Some of the soil was placed in 12-inch pots in the greenhouse and planted with potatoes known to be free from powdery-scab. When the crop had been harvested, which was before all the vines were dead, and the root systems carefully washed so that they might be examined for any signs of infection, roots of all sizes were found to be very generally infected with white galls (Pl. 7, fig. C, D) strongly resembling the well-known legume nodules. On sectioning these galls (Pl. 8, fig. A) when nearly mature, they were found to contain a large number of immature spore balls of *S. subterranea*. Similar galls were found on the stolons and main stems of the plant (Pl. 7, fig. A, B), and in two of the pot cultures pustules were found on the stems about 1½ inches above the surface of the soil, this latter infection having taken place probably while the soil covered this portion of the stem.

In one case in which the galls on a single plant were counted, 149 were found on the roots, 19 on nine stolons, and 8 on three stems; besides, some were doubtless lost in the process of disentangling the roots. This plant produced nine tubers, four infected, and five free from *Spongospora subterranea*.

The presence of *Spongospora subterranea* on the roots of potato plants growing in pot cultures in the greenhouse naturally raised the question as to whether infection is as prevalent under field conditions. Careful watch was kept for evidence of the disease on the roots of plants growing in the soil-treated plots on infected soil at Caribou during the summer of 1915. The first infection was not found until August 5, although examinations had been made weekly from the time the plants began to come up. At this time the galls, which were on the small rootlets both near the surface and deep down in the ground, were white and no larger than pinheads; but day by day they became more pronounced until finally they were comparable to those found in the greenhouse. No infections were found on the tubers when first noted on the roots,

although over 200, many as large as hen's eggs, from 47 hills were examined.

On August 11, 100 hills were dug from one row, and on examination galls were found on the roots in every case, and in many instances were more numerous than on the roots of the plants grown in the greenhouse. From the progeny of these hundred hills five tubers were found that showed under the epidermis of the tuber early stages of infection with *Spongospora subterranea*, consisting of purplish brown, fimbriate, bacterial-colony-like spots, some no larger than pinheads. Infections on the roots progressed more rapidly than on the tubers, as shown by the foregoing statements, but whether this was due to the former's having been infected earlier or whether the fungus developed faster on the rootlets is not known.

It is not unusual—in fact, it is very common—for the root system of a potato plant to be quite generally infected while the tubers remain totally free. This occurred in the case of the Green Mountain and Irish Cobbler varieties, which are generally grown in the vicinity of Caribou. It was very common in the variety plots to find the root system badly infected and the tubers absolutely clean. This leads to the natural conclusion that the critical test for the presence of the disease in a field or section is the freedom of the root system, and that the roots and not the tubers are the organs of the plant which determine the resistance of potato plants to the disease.

The development of new galls on the roots in the field ceased before the vines died or were killed by frost. There was an outbreak of root infection on August 5, and this lasted about two weeks; but after that no new galls could be found, and those present matured and broke up into a mass of spore balls of *Spongospora subterranea* in exactly the same way as does the content of the sorus on the tuber. Why new infections did not continue to develop is not well understood, as there were plenty of young tender rootlets and the soil conditions were apparently comparable with those which existed earlier. It may well be that there are host relations and environmental influences that check the disease after it has reached a given stage of development or time of the year.

When infected seed was planted in virgin soil, root infection took place in 57 days; where clean seed was planted in infested soil, it took place in 69 days. The first galls found were on rootlets close to the diseased parent tuber, but later others showed infection. Although not as numerous on the plants grown in infected soil, it was not uncommon to find from 30 to 50 galls on a single plant grown in virgin soil. The periods of infection mentioned do not, of course, represent the incubation period of the disease, because of the fact that some time elapsed before the plants threw out roots. A better idea of the time required for infection and development sufficient for galls to become conspicuous can be formed from the following experiment.

Twelve plants with strong root systems and from 8 to 10 inches high were washed and transplanted from clean to infected land in the field on August 5, the day on which root infection was first discovered, and for three or four days they were well watered and shaded. On August 19 three of the plants were taken up and the roots examined, but only one gall was found. However, when the nine others were dug, on September 3, and the roots examined, from 3 to 11 galls each were found on 6 plants, which suggests that from 14 to 34 days must elapse after the plant reaches a certain stage of development before infection with *Spongospora subterranea* takes place.

A similar experiment was begun on September 7, plants of approximately the same age as those used on August 5 being transplanted from healthy to diseased soil. The plants were dug on October 1 and the root systems and tubers carefully examined, but no infection was found.

The small amount of infection in the case of the first experiment and absence of infection in the second is explained by the fact that very few infections took place on any of the plants on the plots after August 15, as previously noted.

With a view to determine whether the plant must be a certain age before infection can take place, 200 seed pieces were planted on July 26 in infected soil in the field. On August 20, when the plants were 3 inches high and had extensive root systems, 100 hills were dug and examined, but no infection was found, although in an adjoining row planted on May 26 the roots were generally infected, infection having occurred between August 1 and 15. On September 24 the remaining hundred hills were dug and examined, but no infection was found, although doubtless the plants had some roots by August 15, which tends to indicate that the host tissue had not reached the susceptible stage.

Very little is known about the factors that favor infection. Moisture, however, is doubtless an important limiting factor. Lime increases the amount of powdery-scab on the roots and tubers. Root galls were especially large and abundant on the plots that received lime at the rate of 3,000 pounds per acre. That injuries also increase the tendency to infection was indicated by the finding of numerous galls on lesions caused by fungi other than *Spongospora subterranea*. In one case four galls were found on an injured portion of a plant that had been partially broken off and had recovered, the indications being that infection had taken place after the injury.

NEW HOSTS OF SPONGOSPORA SUBTERRANEA

As soon as it was found that *Spongospora subterranea* infects the root system of the potato plant, investigations were undertaken to determine whether it infects the roots of other solanaceous plants. Fifty-three species of solanaceous hosts were planted at Caribou on June 2, 1915, in powdery-scab-infected soil in adjoining beds about 3 feet square, potatoes

having already been planted on three sides of the experimental plot. Of the 53 species planted, only 16 grew; but this is not surprising, in view of the fact that many were from tropical sections of the world, while the summer season at Caribou is distinctly temperate and short.

The solanaceous plants which became infected are:

Solanum warscewiczii.
Solanum haematoclodum.
Solanum mammosum.
Solanum marginatum.
Solanum ciliatum.
Solanum commersoni.
Lycopersicon esculentum.

Those which remained free from infection are:

Solanum nigrum.
Solanum mauritianum.
Solanum duplosumatum.
Solanum labelii.
Solanum heteracanthum.
Solanum seaforthianum.
Solanum laciniatum.
Solanum torvum.
Solanum sp.

As shown by the lists, infection resulted on 7 of the 16 species that grew, and all that grew were species of the genus Solanum except *Lycopersicon esculentum* (tomato).

In the case of *Solanum commersoni*, a tuber-bearing plant very closely related to *S. tuberosum*, the cultivated potato, 50 hills were planted and 46 grew. On August 20 six of these hills were dug and examined. Numerous galls, similar to those on the potato plant, were found on the roots, but the tubers were free from infection, as were also the tubers from the remaining hills when harvested, on October 10.

In the case of *Lycopersicon esculentum*, the seed was sown rather thick; consequently the plants, none of which grew to be more than a foot high, were crowded in the bed and the soil was a solid mass of roots. Infection was first found on August 20, at which time the galls were quite pronounced and were present on roots of all sizes (Pl. 9, fig. B, C). The galls were examined for evidence of immature spore balls; but, in marked contrast to the potato galls, in which immature spore balls were very evident, none were found. Even as late as September 24 the galls contained no spore balls. This is believed to be due to the fact that the host plant continued rapid growth until killed by frost; for as long as the growth of the host continues the fungus penetrates deeper into the tissues, without any marked tendency to form spore balls.

In the case of *Solanum warscewiczii*, a subtropical ornamental plant and probably a native of South America, 22 of the 66 plants growing in the bed were infected. Two very interesting points in the reaction of this host are the large size of the galls and their formation in a ring around the taproot (Pl. 9, fig. A). Although there seemed to be no difference in general vigor between the infected and noninfected plants, nevertheless this girdling of the taproot doubtless interferes materially with the natural processes of the plant. As in the case of *Lycopersicon*

esculentum, no spore balls could be found even after the plants had been killed by frost.

The other four hosts did not do as well as *Lycopersicon esculentum* and *Solanum warscewiczii*, but their root systems were quite extensive and showed numerous galls having superficial characteristics common to those in the potato and tomato. The phloem was vigorously attacked by the parasite, which caused the xylem and vessels to be twisted out of their normal course in many instances, and the cells of the phlœm were hypertrophied and contained a considerable quantity of starch grains.

The most significant fact brought out in these tests is that infection took place in the root system of the tomato, and this is important in view of the fact that the tomato is very extensively grown in the United States, and often on land used for potatoes. The roots are very generally infected and the distortions and malformations more conspicuous and destructive than those on the potato (Pl. 7, fig. C, D).

When *Spongospora subterranea* was found to thrive on hosts other than the potato, examinations were made of the root systems of weeds common in and about potato fields, including members of the Cruciferae, Labiatae, Scrophulariaceae, and many other families, and also of cultivated plants, such as clover, oats, wheat, and barley; but in no case were signs of infection found. No wild species of Solanum were found growing as a weed in the potato fields or in vacant lots near by.

HISTOLOGY OF THE GALLS

The galls on the roots of the potato are simple in structure and may be termed kataplasmatic galls (Pl. 7, fig. C, D). The fungus or exciting agent flourishes only in the phloem or meristematic tissue (Pl. 8, fig. A), as explained by Woronin (17) in the case of rootlets of cabbage seedlings. Occasionally amebæ are seen in the xylem, but they have never been found to be numerous or to show any signs of stimulating the cells to further growth. The plasmodium of the fungus enters the cell and causes marked hypertrophy, which distends the cell until it is from 80 to 140μ in length. Later this divides by cross walls until instead of one large cell there may be as many as six normal-sized cells, all of which have a nucleus and are surrounded by a goodly number of amebæ, which would indicate that there is some sort of reaction between these bodies. Occasionally, however, one or more of the amebæ are distantly removed from the host nucleus. As the gall enlarges, this hyperplastic growth of the phloem tissues often pushes the vascular system out of its normal position. The infected cells always contain numerous large and small starch grains, which do not wholly disappear until the spore balls are mature. Osborn (11) claimed that the fungus feeds on starch; but were this the case, such an abundant supply would not always be present. It seems clear, however, that the fungus stimulates the protoplast to produce

abundant starch. The amebæ finally bunch up around the host nucleus, which disappears before the spore ball is mature. The amebæ vary from 2.5 to 3μ in diameter, are uninucleate and spherical, and contain a single nucleus, which stains heavily with safranin (Pl. 8, fig. A). As in the case of the tuber, the diseased root cells differ from the healthy in that they show a large number of various-sized starch grains.

In *Solanum warscewiczii* the infection is likewise confined to the phloem and the infected cells occur in groups (Pl. 8, fig. B). These groups originate by continual division of one or more cells in both *L. esculentum* (Pl. 8, fig. C) and *S. warscewiczii* (Pl. 8, fig. B), which has been described by Nawaschin (10) for "clubroot" and called by him "Krankheitsherde." However, the starch grains are not as abundant nor as large in the cells of *S. warscewiczii* as in those of the potato or tomato; in fact, numerous infected cells without a trace of starch are often found. The host nucleus in the infected cells does not differ in size from those found in diseased cells, but the cells proper are increased in size and elongate and form the giant cells referred to by Kunkel (6) in the case of the potato. Such cells are also referred to in the case of Ostenfeldiella attacking *Diplanthera wrightii*. Two such giant cells from the potato partially surrounded by smaller healthy cells are shown in Plate 8, figure B. In one of these giant cells it will be noticed that there is only a single cross wall. In another case one of the cells after dividing into three cells showed early stages of another division. Each of the three cells measured approximately 22.3μ in diameter and was almost square, while the cells immediately surrounding these and belonging to the same histological tissue measured only 11.25μ. The amebæ do not differ in size from those in the tomato, and as in the case of that host are found grouped around the nucleus.

It will be noticed that spore balls were not produced on either the tomato or *Solanum warscewiczii*. This is due to the fact that the host plants had not reached maturity before they were killed by frost, the tomato being about 1 foot high and *S. warscewiczii* just blossoming. There can be no question, however, as to the causal agent in either case, as the amebæ in both were uninucleate and showed the same measurements, staining reaction, position in the host cell, and presence in the phloem, which reacts alike in all of the different hosts.

SPONGOSPORA SUBTERRANEA ON THE TUBER

The first evidence of Spongospora infection on the tubers consists of faint, brownish purple, fimbricate, discolored areas about the size of pinheads and resembling in outline very small bacterial colonies (Pl. 10, fig. B). These spots indicate that the causal organism has entered and destroyed the cells immediately under the epidermal layer. At this stage the infection is comparatively superficial; but in from six to eight days the spot may increase in diameter to ½ cm., lose its brownish color, and be replaced by a somewhat jellylike protuberance, consisting of metaplastic

tuber tissue filled with spore balls of the fungus. The diseased tissue gradually dies, disintegrates, and liberates the spore balls, the sorus at this time being mature. The earliest infections on the tuber usually occur about the stem end, but as the host matures pustules may develop about the eye end.

The symptoms do not always agree with the foregoing, but may vary in accordance with external conditions. For instance, it would seem that if a tuber were detached from the mother plant at the time infection is becoming visible and immediately placed in a moist chamber the development of the pustule should continue; but this is not the case, for while the infected area may increase a little, its development is seriously checked. Again, if the stems are cut off at the surface of the soil in the early stages of infection, the formation of a pustule is checked, but the discolored area may increase and the epidermis may be raised slightly, which symptoms suggest that the plasmodium continues to vegetate rather than to break up and form spore balls. Two rows from which the tops were removed on August 11, six days after the first infection was found on one of the plots, showed only 3 per cent infection, while the checks showed 24 per cent, but the former showed a reduction of about 75 per cent in yield. In like manner, if plants are attacked by lateblight shortly after infection with *Spongospora subterranea* begins to appear, further infection is stopped, and that which has already begun makes but little progress. This occurred in many fields in Maine during the season of 1915. The severe outbreak of lateblight checked the development of the potato crop and of powdery-scab. On the other hand, if the potato plant continues to grow after infection has once taken place and external conditions are favorable for the development of *S. subterranea*, the sori increase in depth and diameter (Pl. 10, fig. C), which suggests the canker stage of *S. subterranea* known in Ireland. The tuber shown in Plate 10, figure C, is the progeny of a plant that grew in the greenhouse and did not mature and die down until 152 days after planting, although its tubers showed infection after 76 days. The unusually large size of the sori in this case is attributed to the long growing period of the plant, which was about 40 days longer than usual. Such sori are common where potato plants are grown in wet, infected soil in the greenhouse and would doubtless result in the field if the crop had a longer growing season. In most potato-growing sections along the Atlantic seaboard only about 100 days are allowed, this being especially true in northern Maine.

These greenhouse experiments suggest that the absence of the canker stage in the United States may be due to the short growing period for the potato plant in districts in which *Spongospora subterranea* thrives.

A careful examination of material showing the canker stage as it exists in Ireland[1] (Pl. 10, fig. E) proved that it differs from the prevailing type of scab caused by *Spongospora subterranea* prevalent in this country.

[1] This material was obtained through the kindness of Dr. George H. Pethybridge.

The symptoms are not fully comparable with those produced in the greenhouse, although they had some points in common, as is shown in Plate 10, figures C, D, and E. The diseased surface of this material presented the appearance of having been gnawed and chewed by insects after *S. subterranea* had made its appearance. The wide variation in the symptoms of this disease emphasizes its dependence on environmental conditions.

The resemblance of the injury caused by the flea beetle to that caused by the sori of *Spongospora subterranea* is very striking, as evidenced by the fact that specimens showing the former were received from several pathologists with an inquiry in each case as to whether the injury was due to powdery-scab. There is no doubt that insects play a rôle in the destruction of the diseased portions of the potato plant. The injury probably caused by the flea beetle and other subterraneous larvæ (Pl. 10, fig. A) can be distinguished from that due to *S. subterranea* by the fact that in its earlier stages there is a minute central opening caused by the puncture of the insect, and in its later stages the affected area may show definite splitting, which, however, does not extend very deep into the tissues. Frequently the central portion of the raised area is crumbled, and it is at this stage that the trouble is most often confused with the open pustules of *S. subterranea*. At this stage also it shows another similarity to *S. subterranea*—that is, the sunken area immediately surrounding the sorus, which is due either to what is designated and described later in this paper as physical drying out (Pl. 11, fig. F, H) or to the action of the plasmodium (Pl. 11, fig. D).

In the course of the work the writers also received many specimens showing enlarged lenticels (Pl. 10, fig. F), resembling superficially the early stages of powdery-scab, with an inquiry in each case as to whether the injury was due to *Spongospora subterranea*. Intumescence associated with the lenticels develops when the tubers are held in moist chambers for 10 days. The proliferation of the cells below and around the lenticels at the end of that time resembles the protruding hyperplastic tissue of the sorus in early stages of formation. A microscopic examination and the identification of spore balls within the sorus, however, is the criterion for determining the disease, but, as will be pointed out in the following paragraphs, care should be taken not to confuse the spore form of other fungi with the spore balls of *S. subterranea*.

CONFUSION IN EARLIER WRITINGS DUE TO THE FUNGI ASSOCIATED WITH SPONGOSPORA SUBTERRANEA ON THE POTATO TUBER

Wallroth (15) and Berkeley (1) described fungi associated with a potato disease which bear a striking resemblance to the spore balls of *Spongospora subterranea*, and earlier investigators observed numerous fungus threads in the sori, all of which resulted in much confusion and error. The writers believe that the "bulbils" of a species of Papulospora

which they isolated from the sori of *S. subterranea* were largely responsible for this confusion and error.

Wallroth saw in the spore balls of *Spongospora subterranea* a striking resemblance to the smuts and accordingly described the organism as *Erysibe subterranea*. Berkeley (1) more than 20 years after Wallroth's observations published a drawing of two spore forms in connection with an article on the potato murrain and refers these two forms to the spores of *Tubercinia* sp. Fr., which cause a scab. The spore forms shown in Berkeley's drawings are certainly not the spore balls of *S. subterranea*, as the former show a distinct pedicel, but Berkeley's description of their effect on the tuber is highly suggestive of the latter fungus. The surface of the potato, he says, is "covered with pustules, which at length become cup-shaped and are powdered within with an olive-yellow meal consisting of the spores of a fungus." The drawings and description of the fungus create some doubt as to whether he referred to *S. subterranea*. In view of his illustration and statement that he saw the various stages of growth attached to flocci, it is possible he was working with material which in addition to being infected with *S. subterranea* was contaminated with the "bulbils" of *Papulospora* sp. or with the forms referred to by Horne (3)

The presence of fungus hyphæ in the sorus and the consequent hypothesis that there are two kinds of powdery-scab is shown by the following statement by Johnson (5, p. 172):

> In some Scotch material I examined, hyphæ were clearly present in the scab areas outside of the cellular tissue of the tuber, and though some could be accounted for as the mycelial hyphæ of Rhizoctonia scab there were others not so explicable. The hyphæ are swollen, septate, and branching; their contents abundant and granular. In some case chains or masses of spores may be seen arising from the protoplasmic contents of the hyphæ. Spongy spore-balls, very like those of Spongospora, arise, and ultimately the inclosing walls of the hyphæ disappear and leave the balls lying free. The more external balls lose their compactness and break up into single spores or small groups of spores, so that they form a finer powder than Spongospora, whose spore-balls remain intact to the end. The mode of origin of the spores is not unlike that met with in the *Ustilagineæ*, so far as they have been studied; and it thus appears as if . . . there are two kinds of potato scab characterized by powdery spore-balls, the one with a plasmodium—*Spongospora subterranea* Wallr.—the other a Hyphomycete, possibly one of the Ustilaginaceæ.

Horne (3, p. 377, 380) says:

> If spore-balls are present, they are frequently associated with the hyphæ of various fungi—the association is so close in some instances that it is difficult to convince oneself that the spore-balls are not the reproductive bodies of the fungus. . . . In late stages of the disease, and even in the powdery-scab stage, the spore-balls . . . are frequently intimately associated with the hyphæ of various fungi. The spore-balls appear sometimes to be attached to hyphæ, or hyphæ twine around them and link them together.

These statements are indeed significant and show that extreme care must be taken in identifying the spore balls of *Spongospora subterranea*,

which are never attached to hyphæ. Plate 12, fig. G, shows figures given by Horne of spores of fungi associated with *S. subterranea*, and referred by him to species of Verticillium and Stysanus, and also drawings of the "bulbils" (Pl. 12, fig. F) of *Papulospora* sp. found by the writers; the similarity between these is very striking.

As early as 1883 Eidam (2, p. 411–414) called attention to the similarity between a species of Papulospora and one of the smuts. It is the "bulbils" of *Papulospora* sp. that bear such a striking resemblance to the smuts and also to the spore balls of *Spongospora subterranea*. Hotson (4) published a paper that treats in part of the genus Papulospora and includes a good description of the "bulbil." In this paper he says (p. 299):

> Lastly, among the structures which bear a striking resemblance to bulbils, the peculiar spore-balls of *Spongospora subterranea* (Vallr.) Johnson, should be mentioned; which although they might readily be taken for a species of Papulospora, have been shown to belong to the life-cycle of one of the Mycetozoa.

In view of the recent isolation of *Papulospora* sp. by one of the writers from tubers affected with *Spongospora subterranea* this statement is very significant. A culture of this species of Papulospora submitted to Dr. J. W. Hotson was identified by him as *Papulospora coprophila* (Zukal) Hotson. All inoculations on the potato tuber with this fungus gave negative results.

CONTROL MEASURES

Although it has been known for more than 50 years that *Spongospora subterranea* occurs in most northern and central countries of Europe, very little effort has been concentrated on its control; and such studies as have been made apply only to local conditions in Ireland, where cultural, soil, and weather conditions are markedly different from those in the potato-growing sections of the United States. As soon, therefore, as it was found that the disease had become established in some of the leading potato-growing sections of this country, the study of its control became imperative and was undertaken by the writers along four lines: (1) Early harvesting, (2) seed treatment, (3) varietal response, and (4) soil treatment.

EARLY HARVESTING

That *Spongospora subterranea* develops on the tubers only after they are partially mature has already been shown. Although carefully sought for during the entire season of 1914 in the plots in which infected seed was planted, no infections were found on the crop until August 20; and not until about three weeks later did it become common or conspicuous. The first case of infection in the warehouse at Caribou was found on September 12 by the Maine Potato Inspection Service, which employed

about 50 inspectors. By that time 12 per cent of the crop had been dug and much of it doubtless thereby saved from infection. Were infection to appear as late every season, early harvesting would be a very simple and effective means of avoiding the disease.

The results obtained in 1914 suggested early harvesting as a means of wholly avoiding the disease where the crop was growing on land known to be infected. To test this four rows 16 rods long were planted on infected land in 1915. Two of these rows were harvested on August 15, when about two-thirds mature, or about two weeks earlier than usual, the harvesting season here beginning about September 1 and extending to October 10. The remaining two rows were dug on October 10. The two rows dug in August produced 323 pounds; of this lot 153 tubers were infected. The two dug in October produced 412 pounds, of which 167 tubers were infected. These figures show that practically all the infection in 1915 made its appearance before August 15, and that from the standpoint of control nothing was gained by harvesting on August 15, while 89 pounds of potatoes were lost in the two rows harvested at that time. However, had infection taken place as late in 1915 as it did in 1914, the crop on a given farm might have been harvested before it became infected; consequently it is believed that this line of attack has not been exhausted and that it has greater possibilities than the results obtained in 1915 indicate.

SEED TREATMENT

In the spring of 1914 an experiment to control the disease by disinfecting the seed potatoes badly infected with *Spongospora subterranea* was begun, Green Mountain, the variety commonly grown in northern Maine, being used. The seed was treated as shown in Table VI.

TABLE VI.—*Results of experiments in controlling powdery-scab by seed treatment in 1914*

Plot No.	Treatment.	Hills.				
		Average weight.	Total number.	Number sound.	Number infected.	Percentage of infection.
		Pounds.				
1	Check, infected seed	1.39	200	63	137	68.0
2	Seed wet and rolled in sulphur	1.48	152	85	67	44.0
3	Formaldehyde (1:30), 1½ hours	1.27	164	138	26	15.8
4	Atomic sulphur (5 per cent), 1½ hours	1.15	202	148	54	26.7
5	Formaldehyde (2:30), 1½ hours	1.04	136	116	20	14.7
6	Mercuric chlorid (2:15), 1½ hours	1.05	189	184	4	2.0
7	Check, infected seed	.....	104	92	70	42.0
8	Copper sulphate (5 per cent), 1½ hours	.94	142	137	5	3.5
9	Mercuric chlorid (4:15), 53°–54° C., 5 minutes	.93	75	70	5	6.6
10	Formaldehyde (2:30), 46°–50° C., 5 minutes	.90	55	54	1	1.8
11	Mercuric chlorid (4:15), 44°–45° C., 5 minutes	1.14	75	69	6	8.0
12	Check, healthy seed	1.25	200	198	2	1.0

TABLE VI.—*Results of experiments in controlling powdery-scab by seed treatment in 1914*—Continued

Plot No.	Treatment.	Sound tubers.		Infected tubers.		
		Number.	Weight.	Number.	Weight.	Per cent.
			Pounds.		*Pounds.*	
1	Check, infected seed	1,798	222	531	56.5	22.87
2	Seed wet and rolled in sulphur	1,613	200	221	26.0	12.00
3	Formaldehyde (1:30), 1½ hours	1,613	200	56	9.0	3.30
4	Atomic sulphur (5 per cent), 1½ hours	1,750	217	118	16.5	6.30
5	Formaldehyde (2:30), 1½ hours	1,097	136	42	6.5	3.68
6	Mercuric chlorid (2:15), 1½ hours	1,600	198.5	6	.75	.37
7	Check, infected seed	1,260	155	170	2.4	11.80
8	Copper sulphate (5 per cent), 1½ hours	1,068	132.5	9	1.0	.83
9	Mercuric chlorid (4:15), 53°–54° C., 5 minutes	556	69	7	.75	1.24
10	Formaldehyde (2:30), 46°–50° C., 5 minutes	397	49.25	2	.5	.50
11	Mercuric chlorid (4:15), 44°–45° C., 5 minutes	681	84.5	12	1.5	1.73
12	Check, healthy seed	1,640	204	4	.5	.20

After treatment the seed was spread out to dry on a laboratory floor which had been previously covered with paper. When dry, the potatoes were cut and placed at once in 1-peck sacks that had been previously soaked for three hours in a 1 per cent solution of copper sulphate or in sacks that had never contained potatoes. In this way they were kept apart from other potatoes and other sources of infection until planted, June 13. The seed used in the clean checks was carefully selected from several barrels believed to be free from powdery-scab, treated with the usual strength of mercuric chlorid, dried, cut, and bagged in the manner just described. Except in the case of clean check tubers, which were wholly free, each seed piece bore a considerable number of powdery-scab pustules.

The plot selected for the experiment was an old orchard which sloped gently toward the east and which had been in sod the preceding 10 years. The soil had been ridged up toward the apple trees; and consequently the ground was somewhat uneven. The soil was a black gravelly loam, but too wet and heavy for ideal potato land. A complete commercial fertilizer was used at the rate of 1,200 pounds per acre. Because of the irregularity of the ground and shading from the orchard trees, no attempt was made to record the germination of the seed or the development of the potato vines during the season. The relative position of the plots with reference to each other was the same as in the table, and the crop was harvested on October 15.

As shown by Table VI, the progeny in the plots adjoining plot 11, with the exception of one tuber in each of two hills, was free from infection by *Spongospora subterranea*, which showed that the land was not infected previous to the planting in 1914. Notwithstanding this nominal infection in the healthy check tubers, the amount of powdery-scab that developed on the various plots would represent the relative efficiency of the various treatments if other conditions were equal, which was not the case,

for, as already stated, the land was uneven on account of the ridging, and plots 1 and 2 were at the edge of the orchard and had more sunlight and consequently gave a larger average yield per hill.

The hill was considered a unit, each being harvested separately. The tubers were examined individually, being washed before examination if not clean when taken out of the soil, and a record made of the number of infected and the number of sound tubers in each hill and also of the gross weight of each.

As shown by Table VI, some of the progeny of the checks planted with diseased seed gave from 42 to 68 per cent of infection, the variation depending doubtless on the soil and water conditions in different parts of the field.

In drawing conclusions from the results given in Table VI it must be borne in mind that in each case only a comparatively small number of hills were used and that variation in the soil and moisture contents materially influenced the results. Notwithstanding these facts, however, it is perfectly obvious from the tests that several chemicals have a deleterious effect on the development of *Spongospora subterranea*, the most active being mercuric chlorid and formaldehyde, hot solutions being more effective than cold.

During the season of 1915 the most promising of the experiments made in 1914 were duplicated, the land used being cleared in the spring and put in condition for planting. This land was much lower than that used in 1914 and not so well drained. The soil was a rather heavy gray silt with considerable humus at the surface. The soil of parts of this plot was of the type on which much of the infection in northern Maine occurs. The land was planted by hand on June 10, the seed being handled in the same way as that used in the experiment of 1914. The arrangement of the plots with regard to each other and the results obtained when harvested on October 10 are given in Table VII.

The most striking result of this experiment is the infection of the control plots planted with healthy seed. One of these check plots was on each side of the field, which consisted of about one-fourth of an acre, and one in the middle, the former being numbered 1 and 9 and the latter, in which there were four rows, 18. Plots 1 and 9 received the same treatment as the plots used in the experiment in 1914, but plot 18 received special care in preparation and planting; moreover, the seed used in this plot was rigidly selected, inspected, and treated with double-strength mercuric chlorid. Everything with which it came in contact until planted was also disinfected with mercuric chlorid.

TABLE VII.—*Results of experiments in controlling powdery-scab by seed treatment in 1915*

Plot No.	Treatment.	Hills.				Tubers.			
		Total number.	Number sound.	Number infected.	Percentage of infection.	Total number.	Number sound.	Number infected.	Percentage of infection.
1	Check, clean seed	56	47	9	16.0	282	263	19	6.7
2	Check, infected cut seed	64	22	42	65.6	442	349	93	21.0
3	Formaldehyde (2:30), 1½ hours	66	49	17	25.7	392	364	28	7.1
4	Mercuric chlorid + ethyl alcohol (2:15), 1½ hours; 1,000 c. c. of alcohol in 3½ gallons of water	72	59	13	18.0	363	346	17	4.6
5	Check, infected seed	73	26	47	64.3	416	297	119	28.6
6	Atomic sulphur (5 per cent), 1½ hours	84	39	45	53.5	469	388	81	17.2
7	Mercuric chlorid (4:15), 50° C., 5 minutes	65	55	10	15.3	332	321	11	3.3
8	Check, infected cut seed	56	9	47	83.9	428	304	124	28.9
9	Check, clean seed	73	61	12	16.4	449	431	18	4.0
10	Wet and rolled in sulphur	57	37	20	35.0	366	332	34	9.2
11	Formaldehyde (1:30), 1½ hours	93	82	11	11.8	486	474	12	2.4
12	Check, infected cut seed	71	49	22	31.0	374	329	45	12.0
13	Formaldehyde (2:30), 50° C., 5 minutes	78	72	6	7.7	366	359	7	1.9
14	Mercuric chlorid (1:15), 1½ hours	69	63	6	8.7	386	380	6	1.5
15	Copper sulphate (5 per cent), 1½ hours	49	40	9	18.3	327	300	27	8.2
16	Check, infected whole seed	88	19	69	78.4	780	506	274	35.1
17	Mercuric chlorid (2:15), 1½ hours	100	85	15	15.0	391	370	21	5.3
18	Check, clean seed	138	124	14	10.1	643	629	14	2.1

As will be seen in Table VII, 10.1 per cent of the progeny of the hills in plot 18 became infected, and as in all probability the seed was free from infection and the disease is not indigenous to virgin land, the question arises as to the source of the infection. In the case of the healthy control plots the probable source of infection was the adjoining infected controls, soil water, animal life, and cultural methods being the probable agencies by which the disease was spread. Considering the results from each plot individually and in relation to those in adjoining plots, it would seem reasonable to believe that in the case of the healthy checks adjoining those planted with untreated seed, the latter served as centers of infection. In 8 out of 10 cases more infection occurred in the rows next to the untreated checks than in the rows farther away.

Although the infection of the healthy checks diminishes the value of the seed-treatment experiment, it serves to emphasize the infectiousness of the disease and to some extent indicates the rate and means of spread where conditions are favorable for its growth. In the light of the developments on the healthy plots in 1915 it is easily understood that the two cases of infection in the clean control plots in 1914 were doubtless carried from the infected to the healthy controls.

As in the case of the experiments in 1914, those in 1915 show, in addition to the spread of the organism in the soil, that seed disinfection has a beneficial effect in diminishing the amount of infection and that treatment with mercuric chlorid and formaldehyde are the most effective,

treatments with a hot solution of these for a short time being more efficient than with a cold solution for a longer time.

The relative efficiency of the various disinfectants, according to the data obtained for the two years, is shown in Table VIII.

TABLE VIII.—*Average results in controlling powdery-scab obtained from different disinfectants used in the tests of 1914 and 1915*

Treatment.	Average percentage of hills infected.	Average percentage of tubers infected.
Formaldehyde (2:30), 46°–50° C., 5 minutes	4.75	1.20
Check, clean seed	7.58	2.23
Mercuric chlorid (4:15), 44°–45° C., 5 minutes	8.00	1.73
Mercuric chlorid (2:15), 1½ hours	8.50	2.83
Mercuric chlorid (1:15), 1½ hours	8.70	1.50
Copper sulphate (5 per cent), 1½ hours	10.90	4.50
Mercuric chlorid (4:15), 50°–54° C., 5 minutes	10.95	2.27
Formaldehyde (1:30), 1½ hours	13.80	2.85
Mercuric chlorid + ethyl alcohol (2:15), 1½ hours; 1,000 c. c. of alcohol in 3½ gallons of water	18.00	4.60
Formaldehyde (2:30), 1½ hours	20.20	5.39
Cut and rolled in sulphur	39.50	10.60
Atomic sulphur (5 per cent), 1½ hours	40.10	11.75
Checks, powdery-scab seed	64.60	25.12

VARIETAL RESPONSE

For a number of years the Office of Cotton and Truck Disease Investigations has carried on experiments on varietal susceptibility of potatoes to the lateblight fungus (*Phytophthora infestans*) and in this connection has made an extensive collection of European and American varieties reputed to be more or less resistant to lateblight. The study of powdery-scab also suggested the selection of resistant varieties as a possible means of control, and accordingly it was decided to use the above collection in the experimental plots infected with powdery-scab. In addition to this collection of standard varieties, a collection of seedlings also was used in the experiment.[1]

A piece of ground with a uniform soil type and infected with *Spongospora subterranea* was selected and 25 hills each of the different varieties of seedlings were planted, the Green Mountain variety being planted in alternate hills as controls. Table VIII gives the varieties tested and the results of the tests, including the percentage ratio of infection in the varieties tested and the checks.

[1] These seedlings, which have not yet been distributed to farmers and seedsmen, were obtained through the courtesy of Prof. William Stuart, of Horticultural and Pomological Investigations, Bureau of Plant Industry, by whom they were developed.

TABLE IX.—*Results of tests of potatoes for resistance to powdery-scab in 1915.*

Variety.	Tubers.			Control tubers (Green Mountain).			Infection[a] in terms of p. ct. var. × 100 p. ct. G. M.
	Number planted.	Number infected.	Percentage of infection.	Number planted.	Number infected.	Percentage of infection.	
Eldorado	54	0	0	98	5	4.8	0
Farys	67	0	0	54	12	18.1	0
Prof. Wohltman	60	0	0	53	19	26.3	0
Senator	215	0	0	159	9	5.3	0
Ursus	178	2	1.1	46	15	24.6	4.473
Pearl	256	2	0.7	65	9	12.1	5.785
Aldona	194	4	2.0	44	21	32.3	6.192
Gryf	452	21	4.4	24	9	27.2	16.176
Bonar	93	9	8.8	26	14	35.0	25.143
Cimbals	68	3	4.2	63	9	12.5	33.600
Constantia							
Gracya	323	46	12.4	17	7	29.1	42.612
Gedymin	215	18	7.7	103	17	14.1	54.610
Prof. Wohltman	56	39	41.0	54	39	41.8	98.086
Kalif	193	188	49.3	34	34	50.0	98.600
Topaz	88	12	12.0	144	14	8.8	136.364
Soliman	147	61	29.6	52	11	17.4	170.115

[a] In order to get a direct comparison between the different varieties, the percentage of infection in any variety was divided by the percentage of infection of the Green Mountain variety planted in the same row and the result multiplied by 100.

As shown in Table IX, 4 of the varieties tested escaped the disease, but none of the checks were entirely free, the percentage of infected tubers in the latter varying from 4.8 to 50 per cent. Out of the 16 rows, 3 showed over 15 per cent infection in the case of the varieties tested and 10 in the case of the checks, and the latter fact naturally raised the question as to why the percentage of infection varied so in the checks, all of which were planted to the same variety.

While it may be that the soil was not generally infected or that conditions in small isolated spots were unfavorable for the development of the disease, the fact that there was often a wide difference in the amount of infection in alternate hills of the control and the other variety in the same row is significant. A striking example of this latter is shown in the row planted to Farys and the row of Ursus, the former showing no infection and the latter 1.1 per cent of infection, while the Green Mountain, the control variety planted in alternate hills in these rows, showed 18.1 and 26.6 per cent of infection, respectively. Another interesting reaction was that in the Soliman variety, which showed 29 per cent, while its check showed only 11 per cent of infection.

Thirty different selections of seedlings were planted in infected soil, and the checks in this case also were planted with the Green Mountain variety in alternate hills in each row. Table X gives the details of the experiment.

TABLE X.—*Results of tests of potato seedlings for resistance to powdery-scab in 1915*

Collection No.	Seedlings.			Control tubers (Green Mountain).			Infection in terms of p. ct. seed × 100 p. ct. G. M.
	Number planted.	Number infected.	Percentage of infection.	Number planted.	Number infected.	Percentage of infection.	
1357	79	0	0	130	18	12. 1	0
1488	101	0	0	44	30	40. 5	0
992	98	0	0	52	2	3. 7	0
2892	125	0	0	58	5	7. 9	0
628	99	0	0	36	2	5. 2	0
2315	59	0	0	98	9	8. 4	0
2387	128	0	0	42	4	8. 7	0
2193	90	1	1. 1	21	6	22. 2	4. 955
1522	129	3	2. 2	26	12	31. 5	6. 984
1124	160	7	4. 2	34	26	43. 3	9. 700
22402	151	2	1. 3	148	23	13. 4	9. 702
2426	141	3	3. 0	63	13	17. 1	11. 696
4259	119	5	4. 0	43	20	31. 7	12. 618
1429	174	7	3. 8	28	5	15. 1	15. 166
1055	113	18	13. 7	15	14	48. 2	28. 423
4755	112	19	14. 5	72	52	41. 9	24. 606
3700	113	3	2. 5	54	4	6. 9	36. 232
2294	116	1	0. 8	103	2	1. 9	42. 105
2950	126	37	22. 7	27	19	41. 3	54. 964
1212	109	1	0. 9	194	3	1. 5	60. 000
1034	144	21	12. 7	137	32	18. 9	67. 196
2870	171	20	10. 4	53	8	13. 1	79. 389
13660	105	21	16. 6	42	11	20. 7	80. 193
4927	48	14	22. 5	79	24	23. 3	96. 567
4227	213	88	29. 2	80	27	25. 2	115. 873
9681	36	5	12. 2	95	9	8. 6	141. 861
15284	89	72	44. 7	25	10	28. 5	156. 842
14329	107	117	52. 2	108	53	32. 9	158. 663
13896	110	12	9. 7	46	3	6. 1	159. 016
1449	90	114	55. 8	109	22	16. 8	332. 143

As will be seen by a comparison of Tables IX and X, the response to the disease was very similar in the standard varieties and the seedlings, except that the extremes of infection were greater in the latter, ranging from 0 to 55.8. Seven of the seedlings showed no infection, while the checks were infected in every case, the infection varying from 1.5 to 48.2; or in the rows of the nine cases referred to, from 3.7 to 40.5. Although the tubers of these nine varieties were free, the roots showed a goodly number of galls, which indicates either that the roots and tubers do not resist the disease to the same degree or that the tubers merely escaped the disease.

As already explained, infection in 1915 took place only for about two weeks during the growing season—that is, between August 1 and 15—and in case the tubers had not set by this time or had not reached a susceptible stage, infection was avoided. It was also observed that, so far as the amount of infection is concerned, it made no difference whether the variety was early or late. That there is a close correlation between

the development of the cork cambium and susceptibility is not improbable, and this is strongly indicated by the fact that probably 90 per cent of the pustules on the infected tubers from the experimental plots were about the stem ends rather than the eye ends. While this preponderance of infection about the stem end was true in 1915 on the experimental plots, the condition in this respect in other fields was not determined, and no such preponderance was noted during the previous season, when infection occurred much later, or between August 20 and about September 5. A critical examination of some of the varieties and of the seedlings attacked showed that not only the percentage of tubers infected but also the macroscopic character of the sori differ.

Different varieties of potatoes respond differently to the attacks of the disease, as shown in Plate A, which represents various kinds of sori. It was possible to arrange a series from mature sori, varying from the size of pin pricks to that of 1 or 2 cm. as they occur on such standard varieties as Green Mountain and Irish Cobbler. The reaction of the host showing the smaller sori differed markedly from that of the host showing the larger sori. In some varieties the sorus attains a considerable size before it bursts, in others it breaks open very early, while in still others the infected area corresponding to the sorus shows only discoloration and gives the impression that the plasmodium is spread throughout this discolored area, but at no point does it cause sufficient proliferation of the host cells to form a definite open sorus.

The data regarding the response of the different varieties lead the writers to believe that the response is not due entirely to resistance but rather to the fact that the tubers of certain varieties have escaped infection, and furthermore that a variety which will show much less infection than the Green Mountain may be found.

SOIL TREATMENT

Five acres on one of the farms first cleared in the vicinity of Caribou and said to have been under cultivation for at least 30 years were used in 1915 for the study of effects of soil type and soil treatment on the disease. Because of its close proximity to the village, this land has been cropped rather heavily to potatoes during the previous 15 years.

Topographically the tract occupies the position of a high glacial river terrace sloping from west to east. Along the northeast side and in part forming the northern boundary is a well-marked drainage way or ravine in which considerable erosion has taken place, leaving the soil exceedingly stony and with less fine earth than elsewhere in the field. With the exception of this and a small area at the southwest corner, the slopes are not steep and there is little or no noticeable erosion. In the central part of the field the slope appears inconsiderable, but the instrumental determination showed not less than 5 feet elevation in 100 horizontal feet, a

slope sufficient for the rapid drainage of surface waters. On the tract were found certain fairly well marked differences of color, texture, and other physical characteristics of the soil. These soils, which are here designated by a set of arbitrary numbers, are as follows:[1]

SOIL 1.0.—The surface is grayish brown to ashy gray, depending on the moisture content. The texture is silty loam, fairly friable when dry, but somewhat plastic when wet. Numerous small stones and gravel occur in the soil and subsoil, the latter being a light ashy-gray or mottled gray and brown and rather heavy silty loam. The drainage of this soil is sufficient for surface run-off, but appears to be deficient in the subsoil, probably due to seepage of water from the higher lying upland. This type comprises the greater area of the tract.

SOIL 1.1.—The surface of this soil is light brown and is underlain by a subsoil which is gray and much like that of No. 1.0. The texture is about the same as that of the foregoing type, though the structure of the surface is more open and friable, stone and gravel are not so conspicuous a feature, the surface is more sloping, and the subsoil drainage is freer.

SOIL 1.2.—The color of the surface is slightly darker than that of No. 1.0, though the subsoil is about the same and the texture is a silty loam. In the area in the northeastern corner of the tract the content of small stone and gravel is extremely high, so that the amount of interstitial soil material is considerably reduced. The two small areas on the southeastern corner of the tract are less stony, the percentage being about the same as in soil 1.0. The texture of the subsoil is rather finer than that of the surface and more compact, the color being a dark grayish brown or gray slightly mottled with brown to the depth of 3 feet or more. The soil occupies relative depressions along natural drainage ways, and in the larger area there is considerable erosion. While the surface drainage seems adequate, there is so much seepage apparently that the internal drainage is actually poorer than in soil 1.0.

SOIL 2.0.—This soil is a medium- to light-brown silty loam and contains considerable gravel of a smaller size generally than is common to the other types. The subsoil is reddish brown to yellowish brown, with a somewhat larger amount of small gravel than in the surface. There is no mottling, oxidation being quite uniform in both soil and subsoil. This type differs from all other soils in that there is no gray mottling in the subsoil. The topography is abrupt or rolling and both surface drainage and subdrainage are free.

Figure 1[2] shows the distribution of the soil types on the portion of the field used.

[1] The description of soils here given was very courteously contributed by Mr. J. E. Lapham, Scientist in Soil Survey, Bureau of Soils.

[2] This map was made by Dr. Oswald Schreiner and Messrs. J. E. Lapham and H. L. Westover, of the Bureau of Soils, through whose kindness it is published. It was of great value in interpreting results of the experiments.

As there will be seen from this map, there are five different types of soil, which occur very irregularly in both the upper and the lower block. The diagram superimposed on the map shows the arrangement of the plots, the distribution of *Spongospora subterranea*, the percentage by weight of the progeny infected with the disease, and the yield per acre. The seed used was of the Green Mountain variety and was carefully selected and treated with the usual strength of mercuric chlorid. The plots, which were numbered 1 to 15, inclusive, received treatment as follows:[1]

1.—Sodium nitrate, 20 pounds.
2.—Control, with commercial fertilizer, 150 pounds.
3.—Old horse manure, 2,400 pounds.
4.—Control; no treatment.
5.—New horse manure, 2,400 pounds.
6.—Phosphoric acid, 24 pounds.
7.—Ammonium sulphate, 20 pounds, and phosphoric acid, 24 pounds.
9.—Potassium chlorid, 30 pounds.
10.—Ammonium sulphate, 20 pounds.
11.—Flowers of sulphur in drills, 90 pounds.
12.—Flowers of sulphur broadcast, 90 pounds.
13.—Calcium carbonate, 300 pounds.
1b.—Sodium nitrate, 20 pounds.
2b.—Control with commercial fertilizer, 150 pounds.
4b.—Control; no treatment.
7b.—Ammonium sulphate, 20 pounds, and phosphoric acid, 24 pounds.
13b.—Ammonium sulphate, 20 pounds.
14b.—Flowers of sulphur, 90 pounds.
15b.—Phosphoric acid, 24 pounds.

As will be seen from the foregoing outline of treatments, the effect of the common commercial-fertilizer ingredients, as well as of some of the well-known soil disinfectants, were studied on the plots either with or without commercial fertilizer. The treatments in all the plots were applied with the potato planter and at time of planting, except in the case of those marked "b," which were applied on August 12, a few days after the first infections were noted, and each treatment was duplicated on what was known as the upper and lower blocks.

Seven of the control plots received at the rate of 1,500 pounds of commercial fertilizer per acre, or 150 pounds each, and an equal number received no treatment. In the former the percentage of infection ranged from 15.1 to 35.4 and averaged 24.7 per cent, while the latter showed a greater variation, ranging from 4.9 to 36.5 and averaging 23.4, or a total average of 24.05 for the 14 control plots. None of the treated plots gave as high a percentage of infection as the average of the control plots, and those treated with sulphur at the rate of 900 pounds per acre gave the lowest percentage, the average of the four thus treated being 8.7 per cent.

[1] All plots marked "b" received treatment on August 12.

Lime at the rate of 3,000 pounds per acre increased the amount of infection 13.2 per cent over its nearest control in the upper block, but diminished it as compared with the control plot in the lower block, and in this case also reduced the yield 28.3 bushels per acre. The difference in the reaction of lime on the two blocks, however, was probably due to the difference in the soil types in the two cases, which are plainly shown in figure 1.

When the crop was harvested, every other row in each plot was dug with the machine and the tubers examined and sorted before being picked up. A stake was placed at the last case of infection in each row, which was determined and plotted as shown by the shaded area in figure 1. In the

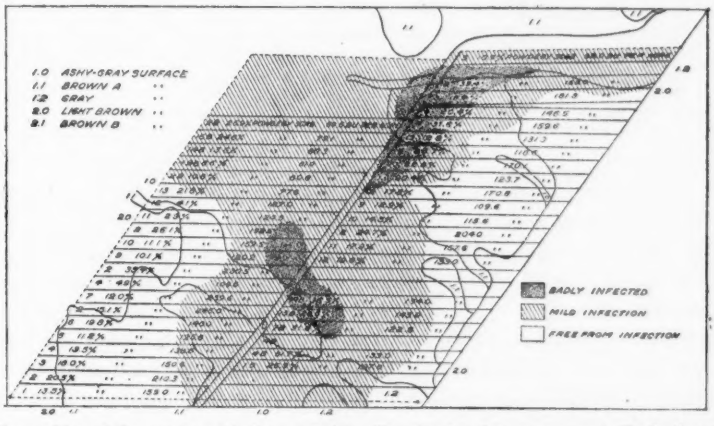

FIG. 1.—Map of the experimental plots at Caribou, Me., showing their arrangement, distribution of *Spongospora subterranea*, percentage by weight of the progeny infected with the disease, and the yield per acre.

lower block the boundary line between the infected and the noninfected portions of the plots was more difficult to mark, the infections being few and scattered south of the lightly-shaded area in the block, especially in the area between plots 7 and 3. The fact that soil 1.1 is partly infected in the corner is doubtless due to seepage from soil 1.0 lying above it.

The significant point brought out in this study is the close relationship of the development of *Spongospora subterranea* to the Washburn silt-loam type of soil, which is marked "1.0" on the map. It was repeatedly observed that where infection was bad the soil had a grayish surface, which showed plainly that it was of the 1.0 silt-loam type. Exceptions to this have been found, but in general there is a close correlation.

Table XI shows the comparative value of the treatments on plots similar in other respects:

TABLE XI.—*Comparative effect of different soil treatments on powdery-scab and on the yield of potatoes*

Plat No.	Treatment.	Percentage of powdery-scab.	Bushels per acre.
1	Sodium nitrate	16.95	138.3
2	Control with commercial fertilizer	24.7	205.8
3	Old horse manure	18.2	165.9
4	Control with no fertilizer	23.8	123.2
5	New horse manure	11.05	108.8
6	Acid phosphate	20.1	143.2
7	Ammonium sulphate and acid phosphate	17.1	204.8
9	Potassium chlorid	11.5	114.9
10	Ammonium sulphate	12.7	137.5
11	Sulphur drill	10.1	140.9
12	Sulphur broadcast	7.35	130.0
13	Calcium carbonate	17.3	104.4

As shown by Table XI, ammonium sulphate and acid phosphate gave nearly the same yield as the checks fertilized, and in addition diminished the amount of infection by *Spongospora subterranea* 7.6 per cent. In the plots treated with potassium chlorid there was less infection than in any of the plats receiving other fertilizer ingredients, or only 11.5 per cent. This is attributed to the slow growth of the plants in the early part of the season and their continued growth until killed by frost. The potassium chlorid apparently prolonged the growing season, and it may be that the crop in a measure escaped the infection period, which, as already shown, was in August.

While these experiments extended through only one season and consequently only tentative conclusions can be drawn, they demonstrated that sulphur at the rate of 900 pounds per acre applied broadcast reduced the amount of infection by *Spongospora subterranea*, and all of the fertilizer ingredients tested reduced the amount of infection from 5 to 12 per cent when applied alone.

DRY-ROT ASSOCIATED WITH SPONGOSPORA SUBTERRANEA

Although *Spongospora subterranea* has been known in Europe since early in the forties of the last century, no mention has been made of a dryrot commonly associated with and following the disease. This rot differs markedly from the many rots which have already been described and which are common to the potato tuber, and a discussion of it necessarily involves the description of several types of rot not heretofore distinguished and a study of their causes. This is the rot to which the senior writer in his brief mention (7) of the shriveling and shrinking

which occur around some of the sori, and the importance of the part played in this connection by the wound parasites which enter through the injury caused by *S. subterranea*. In view of the fact that the rot was first found associated with *S. subterranea* and is most common on potatoes infected with the disease caused by that organism, it seems desirable to call it "powdery-scab dryrot."

HISTORY AND DISTRIBUTION

Powdery-scab dryrot was first observed by the senior writer on potatoes infected with *Spongospora subterranea* collected in New Brunswick, Canada, in the fall of 1913, and held in storage in Washington, D. C. Later it was collected in Aroostook County, Me., and in the infected section of New York State.

Although first found in America, the rot is not confined to this country. A typical case of it was found in a shipment from Ireland by Mr. H. B. Shaw, Pathological Inspector of the Port of New York. About a bushel of the infected tubers collected and sent by Mr. Shaw to one of the writers for examination as to the causal organism showed pronounced discoloration and shrinkage and later on a rot. In the fall of 1913 three 1-barrel sacks were taken at the port of New York from a shipment of potatoes from the Netherlands which showed a considerable percentage of infection by *Spongospora subterranea* and was shipped to Washington, where the diseased tubers were separated from the healthy ones and both lots placed in storage. When these were examined two months later, 21 per cent showed shrinkage and rotten spots.

A dryrot has been found on infected tubers collected in Chile, South America, but in this case the rot was not marked, as in the case of the European tubers, and may well have been accentuated by the long period of transit under poor storage conditions. Be this as it may, however, it is perfectly clear that to some extent at least a dryrot following infection by *Spongospora subterranea* develops on potatoes, no matter where they are grown.

PREVALENCE OF AND LOSSES FROM POWDERY-SCAB DRYROT

Powdery-scab dryrot develops on potatoes after they have been held in storage for some time. It is accelerated by poor storage conditions, but even in good storage from 30 to 75 per cent of the tubers become partially or wholly decayed and consequently worthless for seed or table use. Morse (9) refers very briefly to a rot connected with *Spongospora subterranea* which is doubtless the same as the one under consideration here. This rot, he states, develops on potatoes held in good storage and is hastened when infected potatoes are subjected to ordinary room temperature for a few days.

While it is not an uncommon thing to find powdery-scab-infected tubers entirely decayed, the rot generally occurs in spots. The spots vary from 1 to 10 cm. in diameter. They may be only slight depressions in the superficial layers, or they may extend into the center of the tuber. In this respect powdery-scab differs markedly from the scab caused by *Oospora scabies*, with which it may be easily confused before the dryrot sets in. The rotting of the tubers following infection by *S. subterranea* not only distinguishes this disease from that caused from *O. scabies*, but emphasizes its more destructive nature.

Some idea of the prevalence and destructiveness of powdery-scab dry-rot can be formed from the percentage of this rot on tubers grown during the season of 1913 infected by *Spongospora subterranea*. Infected potatoes were collected from individual growers at different points in Aroostook County between May 2 and June 15, 1914. These were washed and separated into two lots, those showing dryrot and those free from the rot but infected with powdery-scab. Later the lots were weighed, and the percentage proportion by weight of those showing the rot is given in Table XII, together with other data regarding the tubers used.

TABLE XII.—*Percentage of powdery-scab dryrot on potatoes of the 1913 crop infected with Spongospora subterranea*

Date of collection.	Place of collection.	Variety.	Quantity collected.	Percentage of powdery-scab dry-rot.
			Bushels.	
May 2	Patten, Me.	Green Mountain.	6	73
May 5	Presque Isle, Me.	Irish Cobbler.	3	30
May 5	Caribou, Me.	Green Mountain.	8	33
May 21	Patten, Me.	do.	5	67
June 10	Caribou, Me.	do.	12	45
June 15	Ashland, Me.	Irish Cobbler, Green Mountain.	5	35

As will be seen from Table XII, nearly 50 per cent of the tubers showed the dry-rot stage, the percentage of rot on the two varieties ranging from 30 to 73.

Although limited, these observations indicate that the Green Mountain is more severely attacked than the Irish Cobbler. This may be due to varietal differences, but it seems more logical to believe that it is due to the fact that the Green Mountain is a later-maturing variety.

The rot was most prevalent on the tubers infected with the scabby stage of *Spongospora subterranea*, and this explains the low percentage found on the two lots collected at Presque Isle and Caribou on May 5. A very high percentage of the tubers badly infected with the scabby stage showed the dryrot in some form. Not only does the scabby stage of powdery-scab mar and deface the tuber and render it objectionable in

the market, but it may be followed by a rot which will render the tuber worthless for either table or seed purposes.

Naturally the question arose as to whether tubers infected with *Spongospora subterranea* harvested while immature will rot more than such tubers harvested when mature; and in order to get light on this phase of the subject, experiments were carried on with the crop of 1914. About two weeks before the harvest season, or on September 9, 91 tubers were dug from the experimental plots and examined. Out of the total number, 67 showed various stages of infection, ranging from a few small, immature pustules to a generous sprinkling with sori, while 24 were free from the disease. After this the diseased tubers were placed in one sack and the healthy tubers in another, and both sacks were placed in a potato-storage cellar, such as is commonly used in northern Maine. On June 15, 1915, both sacks were examined, and it was found that of the 61 tubers in the sack containing the infected potatoes 31 were two-thirds or wholly rotten, 4 had rotten spots from 2 to 4 cm. in diameter, and 32 had rotten spots from 1 to 2 cm. in diameter. With one exception, which was perfectly sound, the sori apparently having corked over perfectly and prevented the entrance of fungi, all the tubers generally infected with sori of *S. subterranea* were rotten. The 32 tubers with small dryrot spots consisted of those which showed few small sori of *S. subterranea*. Of the 24 tubers in the sack containing the healthy potatoes, 3 were two-thirds or wholly rotten, 1 had a rotten spot 2 cm. in diameter, and 20 were sound, except in the case of 2 which were infected with common scab.

In another case 1 bushel of powdery-scab-infected tubers was collected from a field at the beginning of harvest, or on September 24. At this time the tubers were not fully mature, and the sori in most cases had not ruptured the epidermis; but the badly infected specimens showed brownish purple areas. These potatoes, together with others, were placed in storage. When examined for the development of the rot, on June 10, 1915, 63.9 per cent of the tubers were found to be decayed two-thirds or more, and the collection was in such a condition that it could scarcely be handled. In every case the sound tubers were those which showed but little powdery-scab.

From these results it is evident that powdery-scab dryrot becomes much worse on potatoes harvested early than on those harvested after the tubers are fully mature.

In another case 620 tubers, the progeny of 110 hills in a field infected with powdery-scab, were harvested on November 7, 1914, the potatoes being dug by hand. Each tuber was carefully examined, and it was found that 63 per cent of the lot by weight was infected and that the progeny of only two hills was free from the powdery-scab. The healthy tubers, numbering 295, and the tubers infected with *S. subterranea*, numbering 325, were put in separate sacks and placed in storage on November 9, 1914. When taken out and examined, on June 14, 1915,

only 32 of the sound tubers, or about 11 per cent, showed signs of decay, while 188 of the infected tubers, or about 58 per cent, showed the rot. About 3 per cent of the former and about 58 per cent of the latter were worthless for table use.

Although interesting, the data gathered from the crop of 1913 was not definite, owing to the fact that the conditions under which the potatoes were grown and stored were unknown. More exact data were obtained from the crop of 1914, which was grown on 15 acres of land located in a field in which 75 per cent of the hills had produced tubers infected with *Spongospora subterranea*. From this 15-acre plot 16 barrels were collected on October 7, as they were being harvested, and placed in good storage at Caribou the following day. The amount of infection shown by these potatoes varied from only a few sori to enough literally to cover the tuber. On June 15, 1915, parts of 7 of the 16 barrels were sorted over, and the potatoes showing powdery-scab but no rot were separated from those showing rot. Table XIV gives the results of this study.

TABLE XIV.—*Occurrence of powdery-scab dryrot on the crop of 1914*

Barrel No.	Number of bushels examined.	Number of tubers free from powdery-scab.	Number of tubers showing powdery-scab.	Percentage of tubers showing powdery-scab.
1	1	70	124	63.9
2	2½	170	173	50.1
3	2½	190	368	65.9
4	1	92	124	59.6
5	1½	238	149	40.1
6	1½	38	175	55.9

As will be seen from Table XIV, from 38 to 65.9 per cent of the tubers infected with powdery-scab rotted to such an extent as to be unfit for table use, and this notwithstanding the fact that these potatoes were harvested when fully mature and placed in good storage at once, as already stated.

SYMPTOMS OF POWDERY-SCAB DRYROT

The relation of *Spongospora subterranea* to powdery-scab dryrot is in a way comparable to the rot which follows *Phytophthora infestans*. *S. subterranea* causes little rot, but it leaves an open wound, through which wound parasites may enter. It is well known that *P. infestans* causes little rot unless it is followed by bacteria and wound parasites.

The symptoms of the dryrot following infection by *Spongospora subterranea* may vary greatly, according to the time of year, storage conditions, state of the tuber when harvested, and the stage of development of the sorus when the infected tuber is removed from the soil. In

general, however, the symptoms may be divided into three groups: (1) Desiccation or loss of moisture from the wound caused by *S. subterranea*, (2) those caused by the germination of spore balls in the bottom of the sorus and the formation of the plasmodium which destroys the adjoining cells, and (3) those caused by the entrance of wound parasites into the sori. Often, however, all three causes work together, and in many cases the fungi begin their work after some desiccation and plasmodium injury have set in. It should also be borne in mind that dryrot does not occur about every sorus of a tuber infected with *S. subterranea*. A tuber may be well sprinkled with sori and yet show no dryrot; or the rot may appear about a few of the sori, this latter being true in the case of infected tubers harvested both when immature and when quite ripe and filled with countless spore balls.

Powdery-scab dryrot due to the desiccation of the tissues adjoining the sorus results in discoloration, shriveling, and shrinkage. When infested tubers are harvested, the sori are virtually open wounds, and when placed in warm, dry storage the temperatures incident to which often prevail in early fall, desiccation of the living cells bordering on the wound takes place. When the storage temperature drops with the advance of the season, this type of dryrot is retarded. It can be induced readily, however, by taking tubers from storage and holding them for a fortnight at ordinary room temperature. The cause of the desiccation of the cells about a sorus is readily seen in sections of the sori made before and after shrinkage takes place. Plate 12, figure A, shows the condition in the bottom of a sorus before there is any appreciable amount of desiccation. It should be especially noted that no cork has formed in the bottom of the pit, the adjoining living cells being protected by the dead débris and spore balls of the sorus. By referring to Plate 14, figure A, it will be seen that abundant cork cells are formed in connection with a sorus of common scab. This explains why dryrot is very seldom or never associated with this disease.

A comparison of figures A in Plates 12 and 14 shows clearly why desiccation might take place in one case and not in the other.

The condition of a sorus after some dryrot due to desiccation has taken place is shown in Plate 12, figure B. When the material was dehydrated, the spore balls of the fungus were washed out. It is especially interesting to note the number of dead cells that are still partly intact. There are also signs of the formation of some wound cork. Where this was found, the underlying cells were still alive. There was no evidence in this sorus of a plasmodium, such as occurs in connection with dryrot due to plasmodium injury. The type of dryrot due to desiccation never causes serious damage, but it does further mar the appearance of tubers already injured by the sori of *Spongospora subterranea*.

The second type of dryrot is that caused by the plasmodium of *Spongospora subterranea*. The spore balls in the bottom of the sorus germinate

and form plasmodia, which move about in the immediate vicinity of the sorus and destroy the host cells. This type of dryrot has recently been described by Kunkel (6). The destruction of the cells is followed by a brownish discoloration and shrinkage, which in the early stages is often most conspicuous on one side of the sorus, but later surrounds the pustule and a small depressed area appears (Pl. 11, fig. D). The plasmodium, which has never been found except in the parenchyma, works largely in the superficial tissues, producing a dry, hard spot from ½ to 1 cm. in diameter and ¼ to ½ cm. in depth. Isolations made from this type of rot often give a variety of fungi, most of which are saprophytes; but in some of these isolations wound parasites exist and probably find such injured spots excellent points at which to begin the complete destruction of the tuber.

The entrance of wound parasites through the open sori marks the beginning of the third and most destructive type of powdery-scab dryrot (Pl. 11, fig. B, C, E). In the bottom of many of the sori there is little or no wound cork (Pl. 12, fig. A, B), and the pit is filled with dead tissues and numerous spore balls in the same way as open cavities in the tuber are filled with small masses of culture media on which fungi naturally flourish.

The most common of the wound parasites found associated with the early stages of rot is a species of Phoma, producing brownish to gray lesions (Pl. A and Pl. 11, fig. A). As these lesions progress they become more sunken, darker, and often hard and bony; and when removed, which can often be easily done, they leave a clean and smooth cavity in the tissues of the tuber. The shape and texture of the spots removed give the impression of a button; hence the name "button-rot," by which the trouble is known among farmers. Later stages of the lesions (Pl. 13, fig. A, B) vary from 2 mm. to 5 cm. in diameter and often reach a depth of 2 to 4 cm. (Pl. 11, fig. C). The diseased tissues are sharply defined on the surface, where the pustules are numerous and where infection may take place through each of the pustules; in this way large areas may become discolored and later depressed (Pl. 11, fig. E).

Often, after the above-described lesions have formed, other wound-rot and decay organisms enter, in which case the symptoms are somewhat confusing (Pl. 11, fig. B, C, E). The more common of these organisms are *Fusarium coeruleum* (Lib.) Sacc., *F. discolor*, var. *sulphureum* Schlect., and various bacteria. The symptoms of each of these are typical of the particular species, as described by Wollenweber (16). These fungi have been repeatedly isolated and identified from single-spore cultures as well as from typical rots produced in potato tubers artificially inoculated. Generally when any of these wound-rot organisms are present, the lesion is soggy and less firm to the touch, and its surface is often cracked and broken.

ISOLATION STUDIES

During the past three years a large number of isolations were made from the dryrot lesions, and a considerable number of parasitic and saprophytic organisms, including species of Coniothyrium, Ramularia, Periola, Fusarium, Phoma, Rhizoctonia, Vermicularia, Papulospora, and Bacteria, were studied as regards their relation to dryrot.

A type of spot, which at first had brownish to gray lesions and later became hard and dark (Pl. 13, fig. A, B), was found to be very common, not only in connection with *Spongospora subterranea* but also on tubers free from this disease, the senior writer (7) having found it in Maine before the presence of *S. subterranea* had been reported.

When not associated with any of the fungi above mentioned, the rot caused by the species of Phoma under consideration in section is slate-colored, dry, and powdery; but when other fungi are associated the tissues of the tuber show cavities or chambers and the color is characteristic of the mycelium or spores of the fungus present—that is, white and blue in the case of *Fusarium coeruleum*, light green or sulphur-colored in the case of *F. discolor*, var. *sulphureum*, and dark brown when *Papulospora coprophila* is the secondary saprophyte. The following studies indicate the distinctive characteristics of the rot caused by the species of Phoma under consideration.

Twenty tubers showing typical symptoms were selected for these studies. These were washed and immersed in mercuric chlorid (1:1,000) for 10 minutes, after which the surface tissue was peeled off and plantings made from the newly exposed tissue. In the majority of cases the plantings gave a pure culture of a species of Phoma. The isolations were made from button-shaped spots associated with and apart from *Spongospora subterranea*, and similar results were obtained in each case.

In order to get more accurate data regarding the association of the fungus with the lesion, a more detailed study was made. Twenty tubers having typical lesions were selected on May 17, 1915, an effort being made to secure tubers in which no other wound fungi had entered. The lesions, which varied from 6 to 25 mm. in diameter, were firm to the touch, dark gray, and had the appearance of typical button-shaped spots. The isolation from each tuber was made on a separate plate, and 4 plantings were made on each plate, or a total of 80. These were examined on May 20, and the number and kind of colonies found on each are shown in Table XIV.

As shown by Table XIV, the 80 plantings produced 6 bacterial and 46 fungus colonies. Microscopic examination showed that the latter included at least three different groups of fungi: One containing 2 colonies, one 38 colonies, and one 6 colonies.

In order to check up the identity of these colonies, 2 transfers were made from each of the 46 colonies, to test tubes of potato hard agar and

of sterilized sweet-clover stems. The results obtained confirmed the examinations made direct from the plates. The 38 colonies on both the agar and the sterilized sweet clover in all the test tubes proved to be a species of Phoma. Inoculations were also made with one or more of the fungi from each group, which showed that only the fungus included in the 38 colonies was pathogenic.

TABLE XIV.—*Results of isolations from tubers showing typical lesions caused by the rot of Phoma sp.*

Plate No.	Number of colonies growing on plate.	Number of growths produced on plate.	
		Bacterial.	Fungus.
1	4	0	4
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	4	0	4
7	2	0	2
8	3	0	3
9	1	0	1
10	4	2	2
11	2	0	2
12	2	0	2
13	4	0	4
14	4	0	4
15	4	0	4
16	4	0	4
17	3	0	3
18	3	3	0
19	4	0	4
20	4	1	3
Total	52	6	46

INOCULATION OF TUBERS WITH PHOMA SP.

As the fungus was obtained from the tuber, a series of inoculations was made on tubers, which were selected, washed, and immersed in mercuric chlorid (1:1,000) for 10 minutes, or in some cases in 85 per cent alcohol and burned over the surface. By means of a flamed scalpel the tuber was cut to a depth of 1 to 3 cm., a piece of a pure culture from a transfer 4 days old inserted, and the point of inoculation marked with india ink, after which the potatoes were placed in a moist chamber at ordinary room temperatures. At the end of four days the first indications of the disease became apparent. Ten days after inoculation the lesions reached 6 mm. in diameter. The diseased spots developed until they were from 12 to 25 mm. in diameter, but after this made little or no progress. The control tubers, treated in a similar manner but with no fungus inserted in the wound, remained healthy throughout the entire experiment. The writers have

never been able to produce larger lesions, which is significant, as in nature larger lesions are rarely found unless other wound parasites have entered. A tuber from one series of inoculations in which lesions are only 6 mm. in diameter are shown in Plate 13, figure E, and a series which produced much larger lesions in Plate 13, figure C, and for comparison tubers naturally infected are shown in Plate 13, figures A and B, and Plate 11, figure A. A tuber injured but not inoculated is also shown in Plate 13, figure D.

Table XV gives the results of a series of inoculations made during the course of the work.

TABLE XV.—*Results of inoculating potato tubers with Phoma sp. in 1915*

Date of inoculation.	Number of inoculations.	Percentage of infection.	Condition of control.
March 26	18	88	Healthy.
April 3	18	100	Do.
April 13	6	66	Do.
April 23	24	90	Do.
May 1	6	66⅔	Do.
May 10	9	66⅔	Do.
May 15	8	50	Do.
May 27	10	90	Do.

In the series of inoculations made on May 1 new potatoes were used. The fungus was able to produce very slight infection on these new potatoes, but the lesion never exceeded 3 mm. in diameter. The tubers inoculated May 10 showed only a slight infection. These tubers were held in the moist chamber until May 25, when they were examined. The infected area at this time measured 3 mm. in diameter, and in one inoculation scattered pycnidia could be seen with the naked eye and were as abundant near the edge of the spot as in the center. Reisolations were made on poured plates of potato agar from the inoculations made on March 26, April 3, and May 27, and in every case a pure culture of a fungus, macroscopically and microscopically identical with the culture used for inoculation, was obtained. Plate 14, fig. B, shows a section of one of the inoculated tubers. Most of the mycelium of *Phoma tuberosa* is beneath the epidermal cells. This mat later gives rise to the pycnidia, which grow towards the surface of the tuber.

CULTURAL CHARACTERISTICS

Tests made with the fungus on a number of media showed that it grows well on sterilized sweet-clover (*Melilotus alba*) stems, potato hard agar, potato cylinders, corn meal, and Beyerinck's agar. The luxuriance of its growth on all of these leaves no doubt that it is able to grow

on practically all of the common media. Since its growth does not differ in any essential characteristic from that of other species of Phoma, only the following brief cultural characters need to be mentioned: On sweet-clover stems the growth is fluffy, profuse, and white, turning gray with age; the pycnidial development is good, especially when little moisture is in the tube; the pycnospores often ooze to the surface. On potato hard agar its growth is profuse; the mycelial growth becomes evident within three or four days, is whitish at first, and at the end of a week begins to darken to the characteristic gray of *Phoma* spp.; the pycnidial development is very scarce. On potato cylinders the growth is fluffy and white, turning to gray within eight or nine days, the entire culture turning dark after the development of numerous pycnidia. On corn meal there is a profuse mycelial growth, at first light gray, turning darker with age; numerous pycnidia. On Beyerinck's agar the mycelial growth is scarce and white, and this medium is especially favorable for the production of pycnidial bodies (Pl. 12, fig. C, D).

By actual measurements it was found that there is no difference in size between the pycnospores from the host and those produced in culture, those from both sources, with occasional exceptions, varying from 3.7 to 6.00μ in length and 1.8 to 3.7μ in width (Pl. 12, fig. E).

TAXONOMY OF THE FUNGUS

Saccardo lists four species of Phoma (14) as occurring on *Solanum tuberosum: Phoma nebulosa* (Pers.) Mont., *P. eupyrena*, *P. solani* Cook and Harkn., and *P. solanicola* Prill. and Delacr. The original description of *P. solanicola* (13) states that it was found on the stems, but it was impossible to determine from the literature whether the three others were associated with the aerial part of the plant or with the tuber. The measurements for each of these four species and for the one under discussion are given in Table XVI.

TABLE XVI.—*Size of different species of Phoma occurring on Solanum tuberosum*

Species.	Size of pycnidia.	Size of spore.
	μ	μ
Phoma nebulosa	135–145 × 110–115	7–5–3
Phoma eupyrena	250 ("¼ mm.")	4–1½
Phoma solani	Minute.	7–8 × 1¾–2
Phoma solanicola	Minute.	6–7×4
Phoma sp. under consideration	90–160 × 80–160	3. 78–6. 10 × 1. 8–3. 7

Unfortunately it was impossible to obtain authentic material of the four species described. The original descriptions are too meager to enable one to identify a species with any degree of accuracy, but they show that in size of the spores the species differ markedly from the *Phoma* sp. under

consideration. These meager descriptions and difference in size of spores, coupled with the pathogenicity of this fungus on the tuber, led the writers to designate the organism "*Phoma tuberosa*, n. sp."

Phoma tuberosa, n. sp.

Lesions on tubers of *Solanum tuberosum;* brownish to dark gray or black; 6 to 25 mm. in diameter; sunken, membraneous, with an irregular and sharply defined margin. Pycnidia black, generally scattered over entire surface, subcuticular, irregular, subglobose to spherical, majority provided with a single well-defined ostiole, sometimes breaking at several points for exudation of spore mass, varying in size from 80 to 160 by 90 to 160μ. When placed in water the pycnospores are seen to ooze out in a shiny string, which soon breaks up into the individual spores. Pycnospores 1-celled, hyalin, subglobose, 3.7 to 6 by 1.8 to 3.7μ. Hyphæ septate, dark brown in the tissues of the host. A definite stroma absent.

Habitat.—Wound parasite on tubers of *Solanum tuberosum* often associated with the sori of *Spongospora subterranea.* First found as a storage-rot in Maine.

SUMMARY

(1) *Spongospora subterranea* exists in six different potato-growing sections of the United States, all northern except one.

(2) No infections resulted on the progeny of powdery-scab-infected seed potatoes planted in 15 different localities along the Atlantic seaboard. However, 8 lots of soil out of 12 shipped from as many of these localities to northern Maine and planted with infected seed produced a crop showing powdery-scab.

(3) Periods of damp, rainy, and cloudy weather, coupled with poor drainage, favor the development of *S. subterranea.*

(4) Infection develops earlier on the roots than on the tubers. In 1915 in northern Maine 57 days elapsed between planting of infected tubers in virgin soil and the first signs of root infection. Infection on the tubers appears about the stem end first. All underground portions of the potato plant may become infected with *S. subterranea.* Galls are often very numerous on the root system of potato plants growing in infected soil, while the tubers are absolutely free from infection; hence, a clean root system is the criterion for determining the absence of the disease.

(5) It is not unusual to find parts of fields in northern Maine in which some of the progeny of over 90 per cent of the hills are infected with powdery-scab. Several cases were found in which from 50 to 75 per cent of the 1914 crop was infected. It was found that *S. subterranea* may be spread when infected tubers are planted in virgin soil. Cultural practices and soil water are probably the most important agents in spreading the disease.

(6) Besides the potato, there are seven other solanaceous hosts of *S. subterranea,* including the tomato, as determined by the writers. The disease manifests itself on these hosts in the form of large destructive galls on the roots, these being fully as injurious as those on the potato

plant. The histology of the galls on all the hosts is very similar and has many points in common with *Plasmodiophora brassiceae* on cabbage.

(7) The absence of the canker stage of *S. subterranea* in the United States may be due to the short growing period afforded the potato crop in infected districts.

(8) Flea-beetle injury, intumescence associated with the lenticels, and certain forms of common scab on the tuber are often mistaken for stages of *S. subterranea.*

(9) Among the saprophytic fungi found associated with the sori of *S. subterranea* is a species of Papulospora. The "bulbils" of the latter are strikingly similar to the spore balls of the former fungus, and this similarity may account for the confusion in earlier writings as to its identity on the potato tuber.

(10) A study of early harvesting, seed treatment, varietal response, and soil treatment as control measures for the disease was made. This suggests that (a) early harvesting may be beneficial certain seasons in Maine, but can not be relied on every year; (b) seed treatment with certain chemicals will reduce the disease, this being especially true of mercuric chlorid and formaldehyde, the hot solutions for short periods being probably as efficient as the cold for longer periods; (c) certain varieties may escape infection; this may be due not to disease resistance but to differences in development at the time infection is most likely to take place; (d) the possibility of finding a resistant variety has not yet been exhausted; (e) no soil treatment will eradicate the disease, but sulphur at the rate of 900 pounds per acre applied broadcast reduces the amount of infection by *S. subterranea.*

(11) Several types of dryrot follow *S. subterranea.* These, designated according to cause, are desiccation, plasmodium, and wound-parasite injury. The percentage of these secondary rots as found in nature in infected tubers varied from 30 to 73.

(12) There is a close relation between certain soil types and the development of fungus. (See fig. 1.) From the type of soil and its drainage it is possible to predict what the development of the disease will be in any particular field.

(13) The dryrot due to a species of Phoma and other wound parasites is the most serious of the rots. After a comparison with earlier descriptions of various species of Phoma on the potato, the writers designated the species here discussed as "*Phoma tuberosa*, n. sp."

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PLATE A

Spongospora subterranea and *Phoma tuberosa* on *Solanum tuberosum:*

Fig. 1–5.—*Spongospora subterranea* as found on different varieties of the Irish potato. Figure 2 shows the sori on the Green Mountain variety. The sori shown in figures 1, 3, 4, and 5 are from seedling varieties not yet distributed.

Fig. 6, 7.—Stages in the development of dryrot caused by *Phoma tuberosa*. Figure 6 shows a very early stage and figure 7 a stage commonly found in April in storage houses.

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PLATE 7

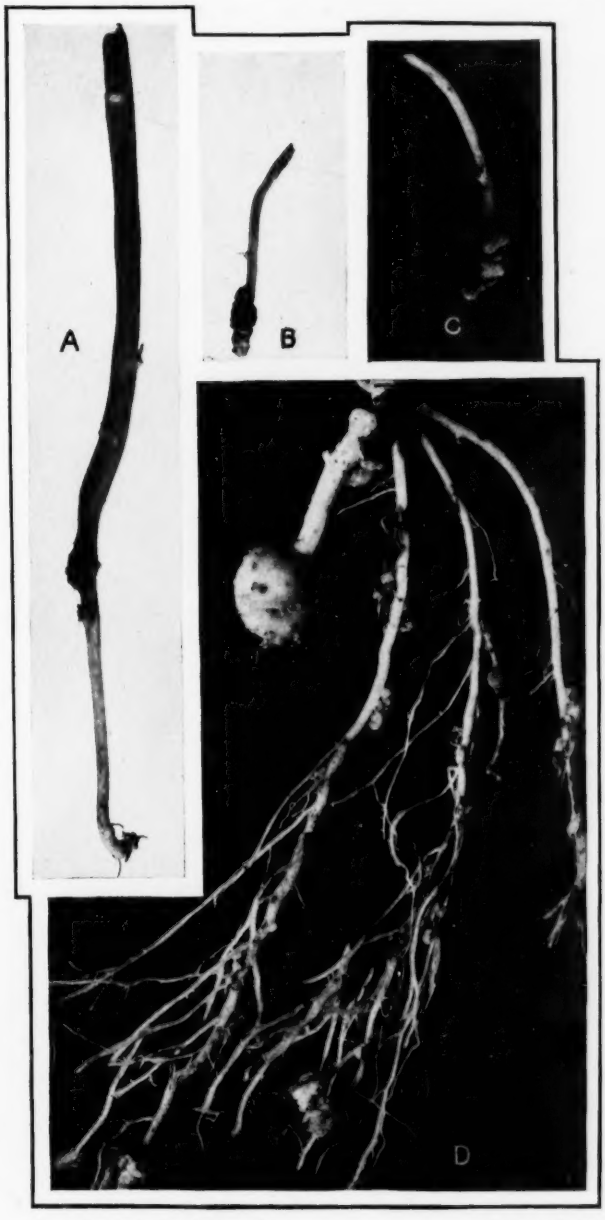
Spongospora subterranea on *Solanum tuberosum:*

Fig. A.—Stem of a potato showing formation of a gall caused by *Spongospora subterranea.* Note the discoloration and shrinkage near the point of formation. Although sometimes present, galls rarely occur on the stems.

Fig. B.—Part of a stolon showing galls caused by *Spongospora subterranea.*

Fig. C.—Discoloration so often found on the root near the point where the galls form.

Fig. D.—*Spongospora subterranea* as found on the root system of the potato. The galls resemble nematode root galls or nitrogen nodules. Generally these galls appear on the roots before any sign of the disease is seen on the tuber.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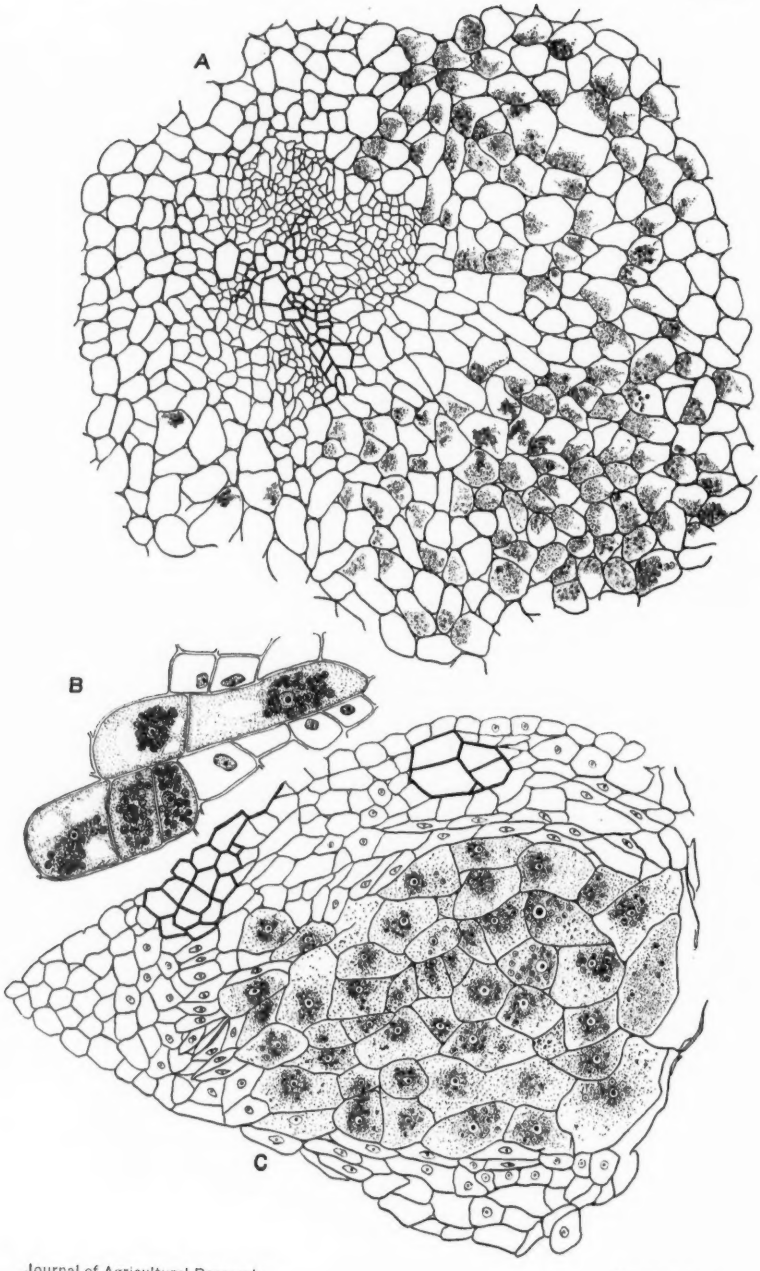
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PLATE 8

Spongospora subterranea in the roots of various hosts (fixed in Flemming's solution and stained with triple stain):

Fig. A.—Section through a potato root affected with *Spongospora subterranea*. The portion to the left is healthy, while that to the right shows the increase in number and size of the host cells. Amebæ in the cells are very numerous. × 110.

Fig. B.—Several cells from *Solanum warscewiczii*, showing the formation of "giant cells" and their division into daughter cells. Note the tendency of the amebæ to cluster around the host nucleus. × 2,600.

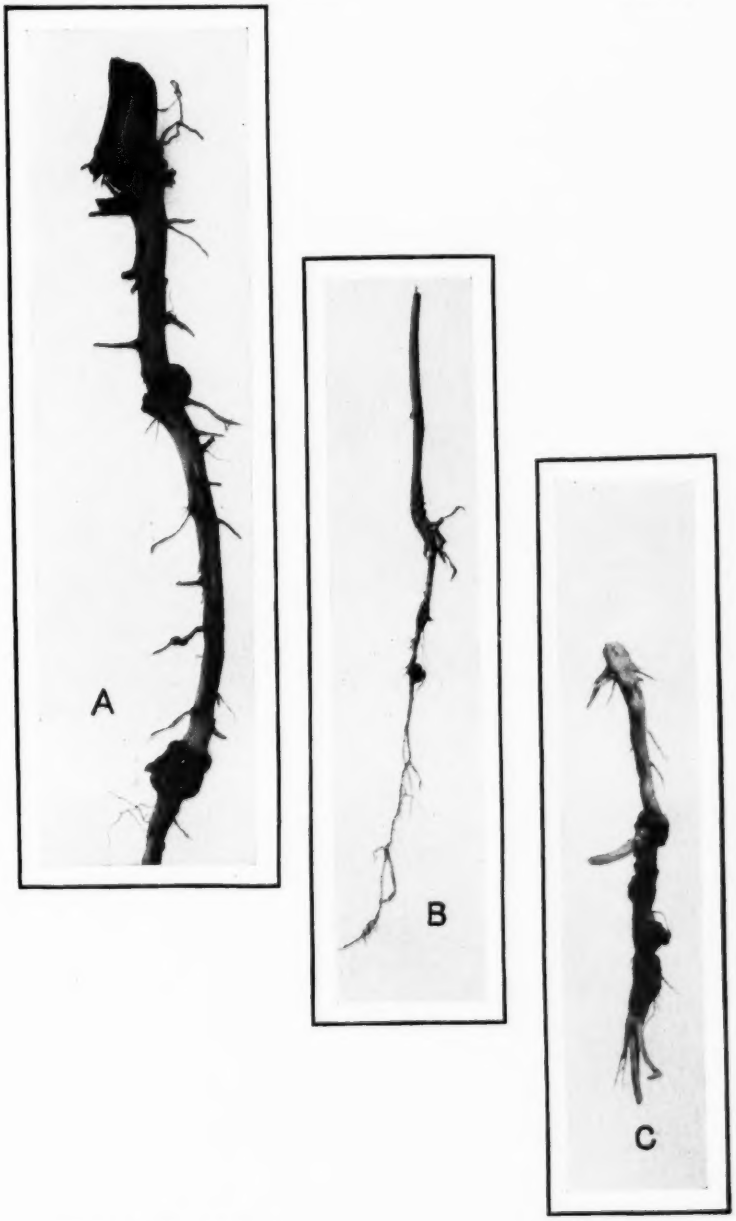
Fig. C.—Section through a tomato root (*Lycopersicon esculentum*, showing effects of infection by *Spongospora subterranea*. Note the abnormal increase in number and size of the cells. The parasite is confined to the cortex, being entirely absent from the xylem. The amebæ are numerous and generally clustered around the host nucleus. × 325.

PLATE 9

Spongospora subterranea on the roots of various hosts:

Fig. A.—Galls caused by *Spongospora subterranea* on the roots of *Solanum warscewiczii*. In this plant the galls have a tendency to girdle the root.

Fig. B, C.—Galls caused by *Spongospora subterranea* formed on the roots of the tomato. These galls, owing to the age and size of the plant, show a variety of forms.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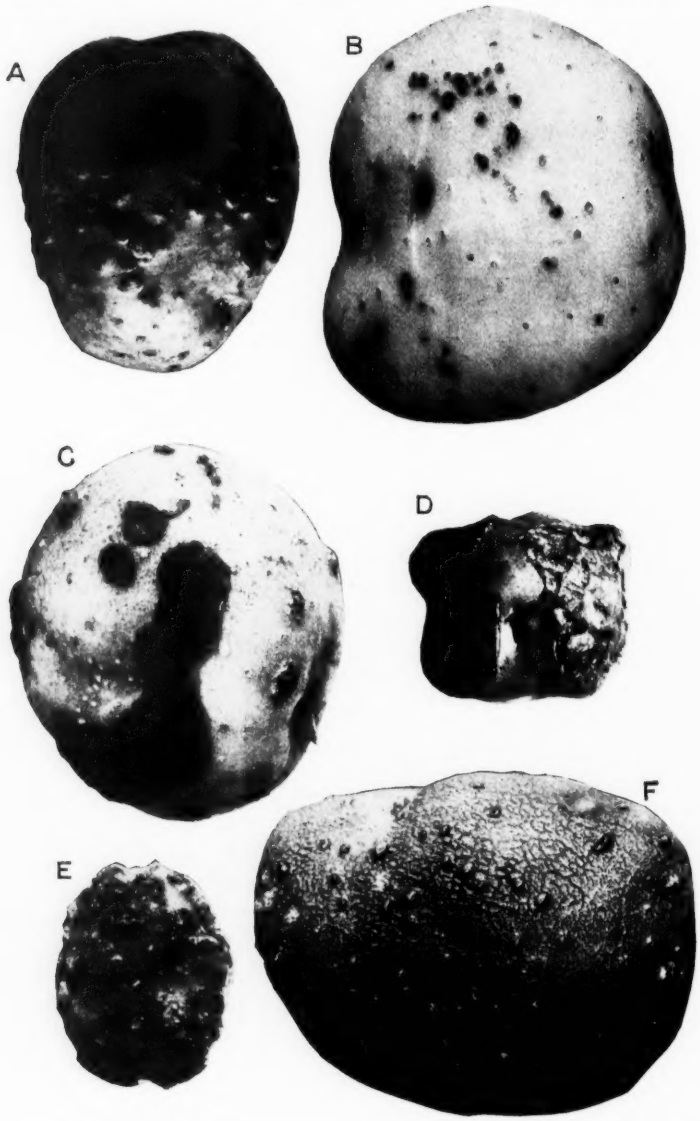
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PLATE 10

Injuries caused by *Spongospora subterranea* and other agencies:

Fig. A.—Tuber showing the effect of flea-beetle injury. Note the similarity of this injury and the young sori of *Spongospora subterranea*. After being placed in storage a shrinkage occurs around the sori-like injuries similar to the desiccation injury around the sori of *Spongospora subterranea*.

Fig. B.—Tuber showing a very early stage of infection by *Spongospora subterranea*. The points of infection show brown, fimbriate colonies. This condition is found in potatoes at time of harvesting. Spore balls are absent.

Fig. C, D.—Tubers grown in infected soil in the greenhouse under exceptionally moist conditions and allowed a long growing season. These lesions resemble very materially the cankerous stage.

Fig. E.—A potato from Ireland showing the cankerous stage. The powdery material is almost gone from the tuber, which appears to have been eaten by insects.

Fig. F.—A tuber showing enlargement of the lenticels. This tuber was kept in a moist chamber for 10 days. Similar enlargements are often found in Florida-grown potatoes, and have also been seen in Delaware and New Jersey. Such conditions are often mistaken for the early stages of infection caused by *Spongospora subterranea*.

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PLATE 11

Dryrots associated with *Spongospora subterranea:*

Fig. A.—A potato tuber showing natural infection with *Phoma* sp. The color of the spot is much darker than that of the healthy tissues. No pycnidia are present as yet.

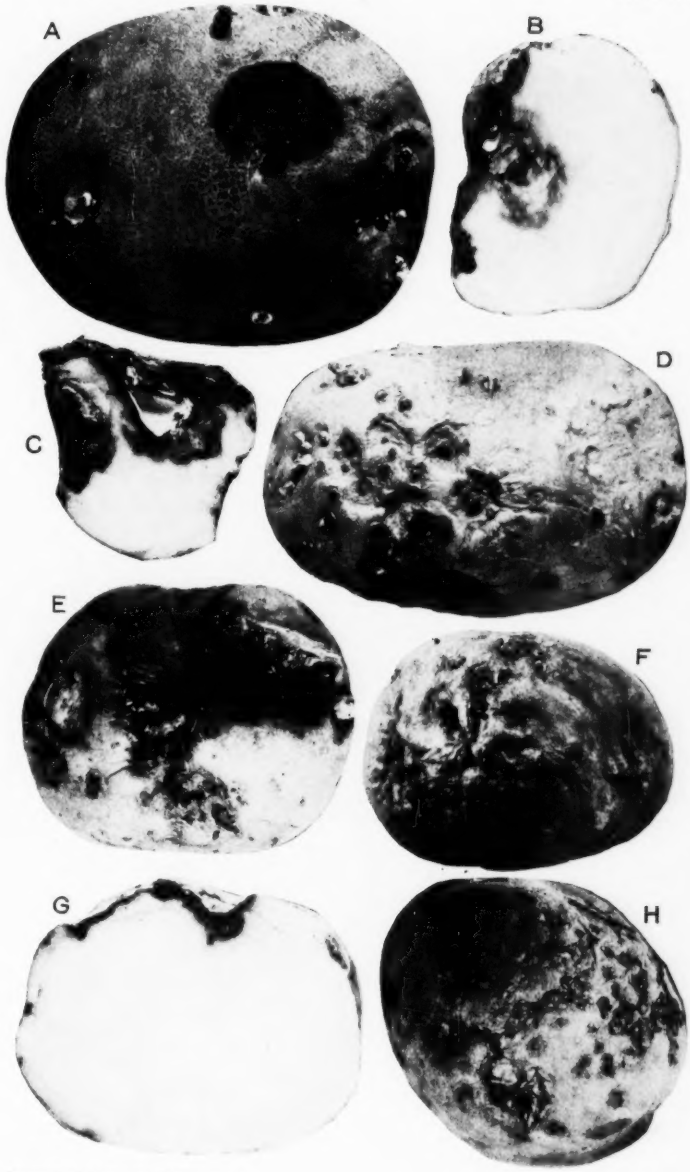
Fig. B, C.—Sections through tubers showing more advanced stages of a rot caused by a species of Phoma. A variety of other fungi have entered, causing further decay of the tuber.

Fig. D.—A potato tuber showing injury immediately around the sori, due partially to the work of the plasmodium. The lower side of the tuber also shows the beginning of the rot caused by *Phoma* sp.

Fig. E.—Infection due to *Phoma* sp. on a potato tuber infected with *Spongospora subterranea*, followed by another, due probably to *Fusarium coeruleum*.

Fig. F, H.—Potato tubers infected with *Spongospora subterranea* about three weeks after harvesting, showing the effects of desiccation injury.

Fig. G.—Section through a tuber, showing the depth to which rot caused by *Phoma* sp. extends. In this case no other fungi had entered.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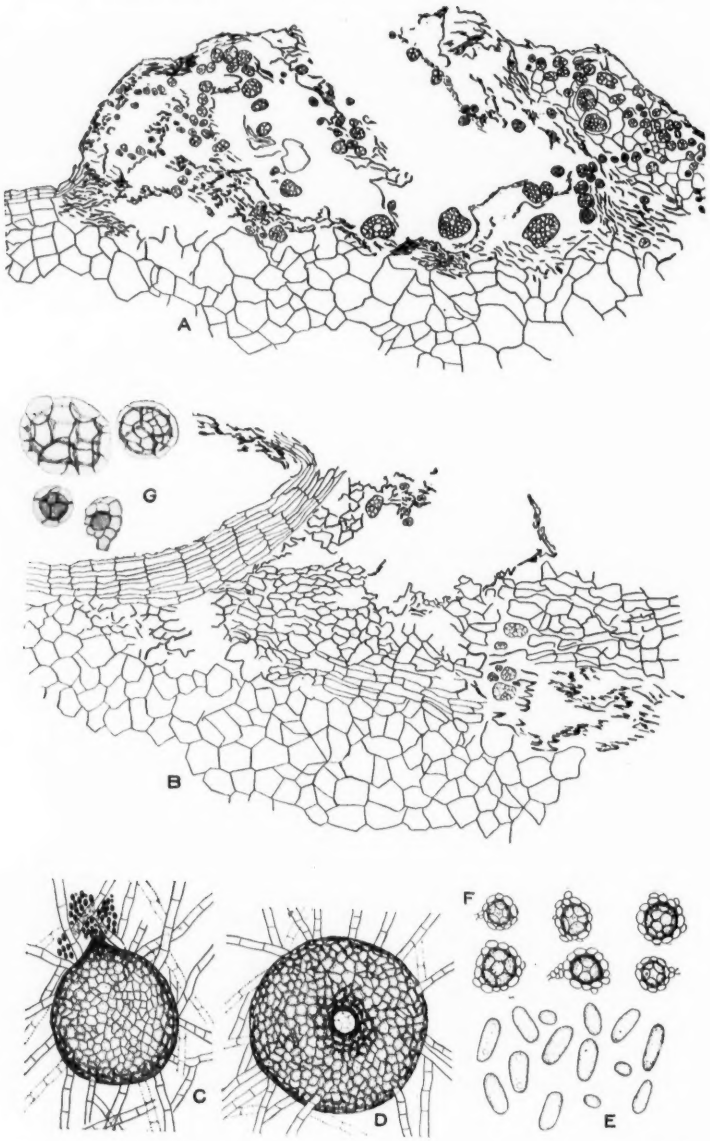
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PLATE 12

Spongospora subterranea and *Phoma tuberosa:*

Fig. A.—Section of a potato tuber through a sorus around which no dryrot has as yet set in. Spore balls are numerous and cork cells absent at the base of the sorus. ×110.

Fig. B.—Section of a potato tuber made through a sorus of *Spongospora subterranea* after the tuber had been held in storage and some dryrot due to desiccation had developed. A tendency toward the laying down of cork at the base is shown, but the cork cells have not yet thickened. ×110.

Fig. C, D.—Two views of the pycnidia of *Phoma tuberosa* as grown in pure culture. Figure C shows the pycnospores emerging from the ill-defined ostiole. ×650.

Fig. E.—Pycnospores. ×2,600.

Fig. F.—Mature "bulbils" of *Papulospora coprophila* (Zukal) Hotson, which in the tissues of potato tubers may be mistaken for spore balls of *Spongospora subterranea*. ×650.

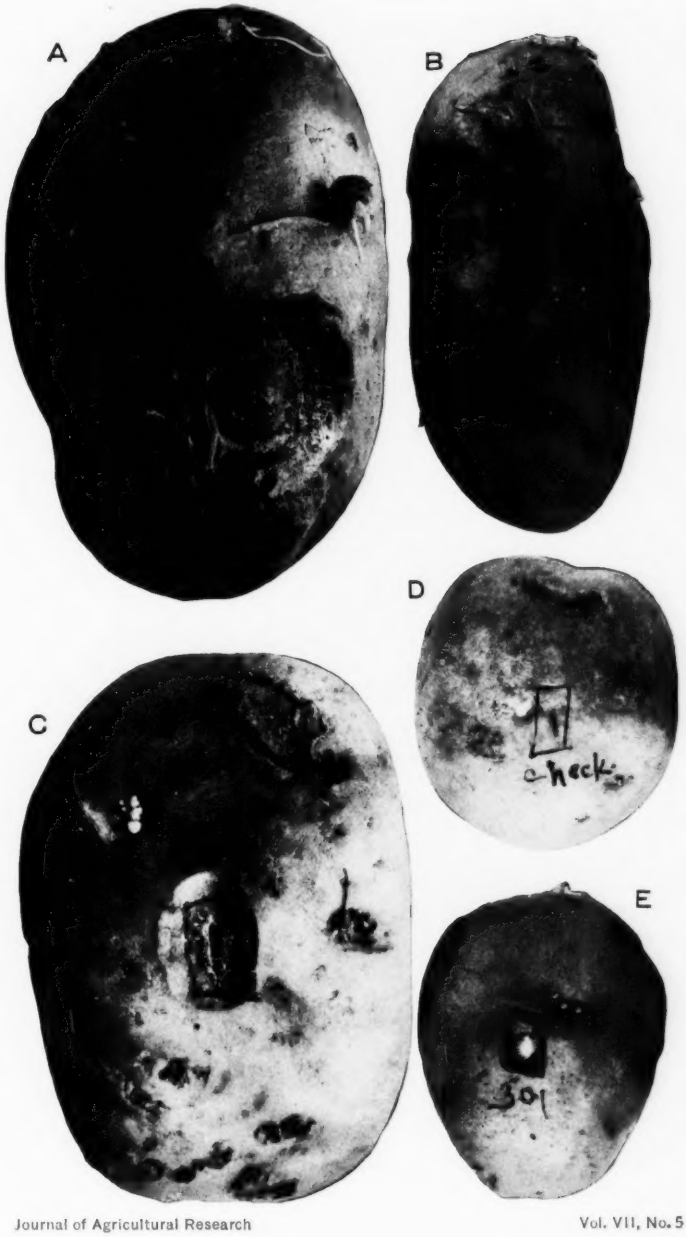
Fig. G.—Spores of fungi associated with *Spongospora subterranea* and referred to *Verticillium* sp. and *Stysanus* sp. by Horne, of whose drawing this figure is a reproduction. Note the similarity to those shown in figure F.

PLATE 13

Phoma tuberosa on *Solanum tuberosum:*

Fig. A, B.—Stages of the rot caused by *Phoma tuberosa* on the Irish potato. The size and appearance of the lesion as shown in figure B is the most common. The abundance and size of the pycnidia vary.

Fig. C, D, E.—Results of artificial inoculation with pure cultures of *Phoma tuberosa*. Figure D shows an injured uninoculated tuber. Figure E shows the buttonhole lesion produced 10 days after inoculation. Figure C shows a tuber from another series three weeks after inoculation. Note the similarity of this lesion and that shown in Plate 12, figure A.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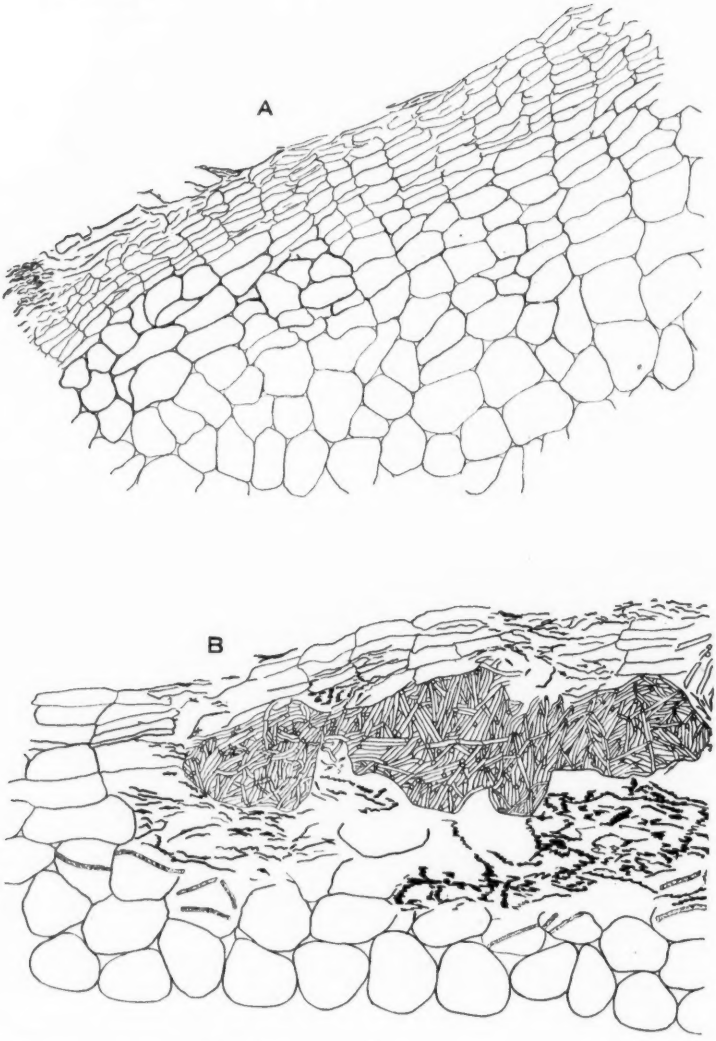
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PLATE 14

Scab caused by *Phoma tuberosa* and *Oospora scabies* on *Solanum tuberosum:*

Fig. A.—Section through a tuber affected with common scab. The portion to the right is healthy tissue. Note the thickened cortex cells formed immediately below the epidermis. These cells prevent the desiccation of the tissues about the scab sorus. Generally such a condition is absent in tubers affected with scab caused by *Spongospora subterranea.* ×325.

Fig. B.—Section through a tuber affected with the rot caused by *Phoma tuberosa.* Note the mat of mycelium immediately below the epidermal cells. It is this mat that gives the lesion its hard, bony texture. The cells below the mat are broken down. It is in this cavity that other fungi begin their work. ×325.

GROWTH OF PARASITIC FUNGI IN CONCENTRATED SOLUTIONS

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The mycelium of a fungus growing parasitically is frequently in contact with the cell sap of its host plant. This cell sap is capable of an osmotic pressure which would vary, of course, with the amount and nature of the compounds in solution. The hyphæ of the invading parasite, then, may grow in a medium which has more or less high osmotic pressure. In order to grow in this medium, they must, of course, be able to withdraw water from it. It is apparent, then, that plant parasites must quite commonly possess the ability to withdraw water from more or less highly concentrated solutions and to grow in them.

The relative concentration of the cell sap of parasite and host has received some attention in the case of phanerogamic parasites. MacDougal (9, 10)[1] and MacDougal and Cannon (11) reached the conclusion that for their "Xeno parasites" the osmotic pressure of the parasite must be greater than the plant into which it is transplanted. Senn (14), in a recent investigation, has shown for certain phanerogamic parasites that the osmotic pressure of the parasite, as measured by the plasmolytic method, is invariably higher than that of its host. This writer seems not to have seen the work of MacDougal. Not so much attention has been paid to fungus parasites, though some work has been done in growing fungi in concentrated solutions. Eschenhagen (4) grew *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea* on rather highly concentrated solutions of various substances. He found that these fungi grew in a saturated solution of potassium nitrate at ordinary temperatures and that the concentration of glucose which limited growth was above 50 per cent. He came to the conclusion that the ability of the fungi to live and grow when transferred to a higher concentration was due to a heightened osmotic pressure within the cell produced by an actual increase in the osmotically active substance therein.

Raciborski (13) also has shown that some fungi can live in exceedingly concentrated solutions. He grew *Torula* sp. in a saturated solution of lithium chlorid and *Aspergillus glaucus* in a similar solution of sodium chlorid. He considered the osmotic pressure in the cell to be greater than that of the outer medium and attempted to calculate the molecular

[1] Reference is made by number to "Literature cited," pp. 259-260.

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weight of a carbohydrate that would produce the necessary osmotic pressure. The present writer (7) germinated the conidia of *Glomerella cingulata* and grew the fungus in concentrations of calcium nitrate, potassium nitrate, and sucrose, which had diffusion tensions of 29.1, 39.3, and 47.3 atmospheres, respectively.

Dorn (3), in his study on the penetration of plant membranes by fungus hyphæ, mentions that there is an osmotic pressure of about 50 atmospheres in fungus hyphæ.

It seems probable from a consideration of the question and from the work that has been done that fungus parasites should be able to live and grow in solutions of a considerably higher concentration than the total concentration of the cell sap of their host plants. It was considered worth while, however, to obtain more evidence on this point. Ten common parasitic fungi were grown in solutions of salts and sugars of rather high concentrations. The total diffusion tensions of the dissolved materials in the expressed juice of some of their host plants was determined by the freezing-point method. The present paper deals with this work.

The fungi[1] studied were *Fusarium radicicola* Wollenw. and *F. oxysporum* Schlecht., two potato-rotting fungi (2); *Plenodomus destruens* Harter, *Diplodia tubericola* (E. and E.) Taub., *Sphaeronema fimbriatum* (E. and H.) Sacc., and *Rhizopus nigricans* Ehrenb., which are parasitic upon sweet potato (6), and *Botrytis cinerea* Pers., *Sclerotinia cinerea* (Bon.) Schröter, and *Sphaeropsis malorum* Peck, well-known parasites on the apple fruit. A strain of *Rhizopus nigricans* Ehrenb., which causes a serious rot of the strawberry (16), was also used. The data on the diffusion tension of the solutions in which *Glomerella cingulata* (Stonem.) S. and v. S. was grown are taken from an earlier paper by the present writer. Several of these fungi are parasitic on more than one of the hosts mentioned. The lowering of the freezing point of the expressed juice of apples (*Malus sylvestris*), sweet potatoes (*Ipomoea batatas*), potatoes (*Solanum tuberosum*), and strawberries (*Fragaria* spp.) was determined.

In the experiments with the fungi two methods were followed for the determination of the highest concentrations of the various substances used in which the fungi could grow. In the one method hanging-drop cultures were made of the spores which had been sown in salt or sugar solution of varying concentrations. These cultures were examined by means of a miscroscope, and the highest concentration in which the germination was apparent was noted. The other method was similar in principle to the one just outlined. In this the procedure was to sow the spores in sterilized tubes of the salt (sodium chlorid) or sugar solution and after about a week determine the growth or lack of growth by observation.

[1] The writer's thanks are due Mr. C. W. Carpenter for cultures of the species of Fusarium, Mr. L. L. Harter for cultures of the fungi from sweet potato, Dr. J. S. Cooley for the apple-rot fungi, and Dr. Neil E. Stevens for cultures of the species of Rhizopus from strawberry.

The spores were germinated and the fungi grown in solutions of calcium nitrate, potassium nitrate, sucrose, and glucose. Concentrated solutions were made of these substances, which were diluted down to the concentration desired in the experiments by the addition of distilled water to which a very little potato extract had been added.

The weight normal method of Morse and Frazer (12) was followed in making up the solutions.

The diffusion tension of the highest concentrations used in which the spores germinated and grew was calculated for the various compounds, taking into account, of course, the ionization of the salts. The data for calculating the percentage of ionization of the two salts were obtained from Jones's tables (8). The calculations of the percentage of ionization give probably only approximate values as the calculations are based on interpolations in most cases, and, moreover, some other substances were present which might influence the dissociation of the salts. The data thus obtained, however, probably offer a better basis for the comparison of the diffusion tensions of the two electrolytes and the two nonelectrolytes used in this study than the molecular concentrations given in the adjoining columns. The diffusion tension of the cane-sugar solutions was calculated from Morse and Frazer's determination of the osmotic pressure of a molecular solution of this substance at 25° C. From their work and from the determinations of Berkeley and Hartley (1) it seems quite probable that the values given are too low. The results obtained in growing fungi in the concentrated solutions of salts and sugars are given in Table I.

TABLE I.—*Highest concentrations (molecular) of calcium nitrate, potassium nitrate, sucrose, and glucose in which the fungi grew and the calculated diffusion tensions in atmospheres of these solutions*

Fungus.	Glucose.		Sucrose.		Potassium nitrate.		Calcium nitrate.	
	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).
Fusarium radicicola	1.6	[a] 38.9	1.8	47.4	1.6	54.5	0.6	27.7
Fusarium oxysporum	1.6	[a] 38.9	1.8	47.4	1.6	54.5	.6	27.7
Plenodomus destruens	2.4	[a] 58.3	1.8	47.4	1.6	54.5	.7	33.6
Sphaeronema fimbriatum	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.4	19.5
Diplodia tubericola	2.6	[a] 63.2	1.6	42.1	1.8	58.8	.7	33.6
Rhizopus nigricans (from strawberry)	1.6	[a] 63.2	.....	.....	.8	27.5	.3	15.9
Rhizopus nigricans (from sweet potato)	1.6	[a] 63.2	1.6	42.1	.8	27.5	.3	15.9
Botrytis cinerea	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.6	27.7
Sclerotinia cinerea	.....	[a] 58.3	.....	.....	1.4	47.6	.6	27.7
Sphaeropsis malorum	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.6	27.7
Glomerella cingulata	.....	.....	.....	41.3	.....	[a] 39.3	.....	29.1

[a] No higher concentrations used.

The freezing points of the expressed juice of apples, sweet potatoes, potatoes, and strawberries were determined by means of a Beckman freezing-point apparatus, and the total diffusion tension for the material in these solutions was calculated from the lowering of the freezing point (15).

The results obtained by the calculation of these data are given in Table II.

TABLE II.—*Diffusion tension in atmospheres of the juice of certain hosts of the fungi studied, as calculated from the lowering of the freezing point*

Host.	Diffusion tension (atmospheres.)
Strawberry	8.27
Apple (Blacktwig)	17.85
Sweet potato (Jersey Big Stem)	10.25
Potato (Irish Cobbler)	6.52

From Table I it is evident that the fungi used in these experiments are able to grow in relatively high concentrations of salts and sugars. The highest diffusion tension of any solution in which growth was evident was in the concentrated solutions of glucose. Growth occurred in all concentrations of this sugar used.

With potassium nitrate growth was inhibited in all cases when the diffusion tension of the solution was about 59 atmospheres; and in only one case was a fungus able to grow in a solution so concentrated. All the fungi except the two strains of *Rhizopus nigricans* and *Glomerella cingulata* grew in concentrations which have a calculated diffusion tension of 47 atmospheres.

The fungi also grew in solutions of sucrose of rather high concentrations, the two strains of *Rhizopus nigricans* and *Diplodia tubericola* being the only fungi which were unable to grow in sucrose solutions having a diffusion tension of 40 atmospheres. It is noticeable that growth in the case of *R. nigricans* is inhibited also at lower concentrations of potassium and calcium nitrates than with the other fungi.

Calcium nitrate inhibited germination and growth always at concentrations considerably lower than those required to produce the same effect with sucrose, glucose, and potassium nitrate. Nevertheless a comparison of the diffusion tensions of the highest concentrations of calcium-nitrate solutions in which the fungi grew and the diffusion tension of the juice of the host plant as calculated from the lowering of the freezing points shows that the parasite is in all cases able to grow in considerably higher concentrations than are present in the cell sap of its host plant. Whether or not the protoplasm of the fungi used is impermeable to the two salts and two sugars used in these experiments

is a question which can not be answered from the data now at hand. Potassium nitrate has been used extensively as a plasmolyzing agent for determining osmotic pressure in plants.

Fischer (5) reaches the conclusion, however, that the protoplasts of bacteria are readily permeable to this salt. True (17) used cane sugar in his work on species of Spirogyra, and it has been used to a considerable extent by other investigators. Calcium nitrate is not commonly used as a plasmolyzing agent.

From the concentrations of glucose which were found to be favorable to the growth of fungi it seems probable that this substance penetrated the protoplasm, perhaps quite readily. It is, of course, probable that all the substances penetrate into the cell to some extent. It is possible that the protoplasm of the fungi here used is readily permeable to the salts and sugars employed in this study and that they can pass into the cell until the concentration within is the same as without. Then the presence within the cell of some substance to which the protoplasm was impermeable would raise the osmotic pressure within above that of the surrounding solution, and the fungus could grow.

The importance of this ability of parasitic fungi to grow in solutions which are capable of exerting a high osmotic pressure is evident. Whether this ability is due to the osmotic pressure in the fungus being originally higher or whether it becomes higher through a diffusion of substance into the hyphæ, or whether there is an actual increase in the osmotically active substances within the cell, as Eschenhagen concludes, or whether other factors than a high osmotic pressure enable fungi to remain turgid and grow in concentrated solutions are questions which need further investigation.

In these experiments in which fungi were grown in solutions of potassium and calcium nitrate, sucrose, and glucose it was found that in every case the fungi grew readily in solutions in which the diffusion tensions were much higher than the total diffusion tensions of the dissolved substances in the juices of their host plants.

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